

**Cytogenetic Studies in Some Species
of Genus Atylosia and Cajanus Cajan (L.) Millsp.**

THESIS

Submitted to the Bundelkhand University, Jhansi for the
degree of Doctor of Philosophy in Botany
(Faculty of Science)

by

Kalpana Srivastava

M. Sc. (Botany)

Plant—Improvement Division

INDIAN GRASSLAND AND FODDER RESEARCH
INSTITUTE JHANSI 284003 (U. P.)

1987



Telex : 330-241
Gram : GHASANUSANDHAN
Phones : 833, 808 OFFICE
886 RES.

Indian Grassland and Fodder Research Institute

PAHUI DAM, JHANSI-GWALIOR ROAD
JHANSI-284003, (U. P.)

Dr. S.N. Tripathi
M.Sc., Ph.D. (B.H.U.),
Scientist S-2 (Genetics & Cytogenetics)
Division of Plant Improvement

D. O. No.

Dated *May 4, 1987*

C E R T I F I C A T E

This is to certify that the thesis entitled,
"Cytogenetic studies in some species of genus Alysicarpus
and Cajanus cajan (L.) Millsp." submitted for the
degree of Doctor of Philosophy of Bundelkhand University,
Jhansi (U.P.), is a record of bonafied research work,
carried out by Km. Kalpana Srivastava, M.Sc. (Botany),
under my guidance and supervision.

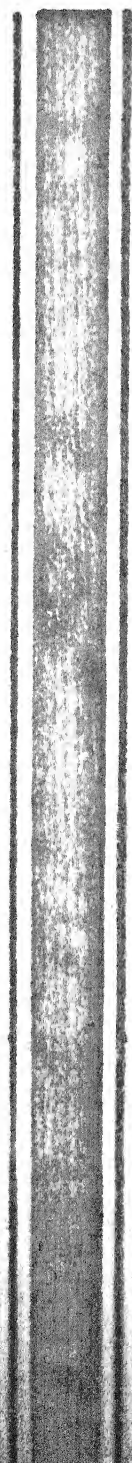
No part of the thesis has been submitted for
any other degree or diploma. All the assistance and
help received during the course of investigation have
been acknowledged.

forwarded
Dangishy L.
DANJAB SINGH
Director
Indian Grassland & Fodder Research Institute
JHANSI

Subhash
(S.N. TRIPATHI)
SUPERVISOR



**DEDICATED IN THE
LOVING MEMORY
OF OUR RESPECTED
DADI AND BAPOO**



ACKNOWLEDGEMENT

It is difficult to express in adequate words my sincere thanks, gratitude and indebtedness to Dr. S.N. Tripathi, Scientist, S-2 (Genetics and Cytogenetics) Plant Improvement Division, Indian Grassland and Fodder Research Institute, Jhansi, for initiating me into this research work, by providing valuable guidance, constant help and encouragement in maintaining the progress of this study. I wish to express my warm feeling of appreciation for Dr. S.N. Tripathi, who was 'kind enough' to make this research work meaningful and interesting.

I am grateful to Dr. B.D. Patil, Ex-Director and Dr. Panjab Singh, Director, Indian Grassland and Fodder Research Institute, Jhansi for making available all the facilities in the Institute for the successful completion of this study.

My sincere thanks are due to Dr. R.B.R. Yadava, Dr. Bhag Mal, Ex-Head of Plant Improvement Division, Dr. S.R. Gupta, Head, Plant Improvement Division and Dr. C.B. Singh, Plant Breeder, for their interest during the course of study.

My thanks are also due to Dr. D.R. Malviya, Scientist, S-1 (Plant Breeding), my colleague,

Miss Suman Parihar, Research scholar (Cytogenetics) and Mrs. Uma Tripathi (W/o Dr. S.N. Tripathi) for their valuable help and co-operation at various stages.

I am thankful to FOTOLAND- Photostudio, Jhansi, for photography and the Librarian, I.A.R.I., New Delhi, for according Library facility.

I am grateful to my parents, brother and sisters for their constant inspiration and support throught the course of investigation.

The author is thankful to Sri. C. Narayan, K.K. Nair and Narayan Singh Rawat for efficient typing of this thesis.

Date. 4..15..1987.

Kalpana Srivastava
(KALPANA SRIVASTAVA)

CONTENTS

| <u>SL. No.</u> | <u>CHAPTER</u> | <u>PAGE</u> |
|----------------|--|-------------|
| 1. | INTRODUCTION | 1-6 |
| 2. | MATERIALS AND METHODS | 6-13 |
| 3. | MORPHOLOGY OF <u>ATYLOSIA</u> SPECIES AND <u>CAJANUS CAJAN</u> | 14-18 |
| 4. | CYTOLOGY OF <u>ATYLOSIA</u> SPECIES AND <u>CAJANUS CAJAN</u> | 19-47 |
| 5. | CROSSABILITY STUDIES | 48-58 |
| 6. | STUDIES ON INTERSPECIFIC HYBRIDS OF- | |
| a) | <u>Atylosia lineata</u> x <u>Atylosia albicans</u> | 59-81 |
| b) | <u>Atylosia albicans</u> x <u>Atylosia cajanifolia</u> | 81-107 |
| c) | <u>Atylosia lineata</u> x <u>Atylosia cajanifolia</u> | 107-130 |
| d) | <u>Atylosia platycarpa</u> x <u>Atylosia mollis</u> | 130-149 |
| 7. | STUDIES ON INTERGENERIC HYBRIDS OF- | |
| a) | <u>Atylosia albicans</u> x <u>Cajanus cajan</u> | 150-173 |
| b) | <u>Atylosia lineata</u> x <u>Cajanus cajan</u> | 174-196 |
| c) | <u>Atylosia scarabaeoides</u> x <u>Cajanus cajan</u> | 197-220 |
| 8. | INDUCTION OF POLYPLOIDY AND STUDY ON INDUCED POLYPLOIDS OF- | |
| a) | <u>Atylosia platycarpa</u> | 221-237 |
| b) | <u>Atylosia albicans</u> | 238-252 |
| c) | <u>Atylosia lineata</u> | 252-268 |
| d) | <u>Atylosia cajanifolia</u> | 268-285 |
| e) | <u>Atylosia volubilis</u> | 285-302 |
| f) | <u>Atylosia scarabaeoides</u> | 302-319 |
| g) | <u>Cajanus cajan</u> | 319-331 |
| 9. | STUDIES ON EFFECTS OF EMS IN- | |
| a) | <u>Atylosia platycarpa</u> | 332-344 |
| b) | <u>Atylosia lineata</u> | 344-356 |
| c) | <u>Atylosia volubilis</u> | 356-368 |
| d) | <u>Atylosia cajanifolia</u> | 368-379 |

Contd..2..

| <u>SL.No.</u> | <u>CHAPTER</u> | <u>PAGE</u> |
|---------------|----------------------------------|-------------|
| e) | <u>Atylosia albicans</u> | 379-393 |
| f) | <u>Atylosia scarabaeoides</u> | 393-406 |
| g) | <u>Cajanus cajan</u> (ICP 8647) | 406-417 |
| h) | <u>Cajanus cajan</u> (SNT Coll.) | 417-428 |
| 10. | GENERAL DISCUSSION | 429-486 |
| 11. | SUMMARY | 487-493 |
| 12. | REFERENCES | |
| 13. | APPENDIX | |

INTRODUCTION

The evolution of desirable plant types to meet new challenges calls for concerted efforts on the assemblance of gene pools and their evaluation. This enables us to understand the extent of genetic divergence for economic traits and to isolate specific types for exploitation in hybridization programme.

The genus Atylosia and Cajanus belongs to family leguminosae (papilionaceae) of the tribe phaseolae and subtribe cajaninae. Genus Atylosia is a widely distributed legume with many species distributed throughout the hemisphere of the world. The wide distribution along various altitudes and latitudes indicates its very high adaptability in different ecological conditions. Species of Atylosia have been suggested a possible source of genetic diversity for traits not detected in Cajanus cajan (Remanandan, 1980). The uses of Cajanus cajan (pigeonpea) are manifold: Dhal, a protein rich dish eaten by most Indians, husks of pods are used as cattle feed, the green foliage as green manure or fodder, dried stalks as fuel, roots for soft coal, the whole plant as a host for the lac insect and foliage for rearing of silk worms, as a soil improver due to its long developed tap roots, cover crop and hedge or wind breaker.

Cajanus as genus was founded in 1813 by A.P. De candolle and Atylosia by W. & A. in 1834.

Cajanus cajan (Linn.) Millsp. was mostly considered to be monotypic genus (Hooker, 1875), because C. kerstingii, Harms described in 1915 from West Africa was unknown to most agricultural scientists. The pigeonpea (Cajanus cajan (L.) Millsp. is now spread pantropically, and is most adapted and productive in the semi-arid tropics and it is a natural assemblage of about 13 closely related genera distributed mainly in tropical regions. The generic distributions are

sometime not based on sharp and well defined morphological characters and biosystematic relationships of these genera are not yet completely understood. The number of species included in each genus varies from flora to flora and author to author. Hooker (1876) included 20 species in Atylosia. Further, Hooker and Jackson (1895) recognised 29 species of Atylosia and showed that out of 29 species of Atylosia 20 are found in India, 5 in Australia and 4 in Burma, Siam and Philipinae. At present, there are 38 species distributed in tropical Asia, Australia, India and Medagaskar. However, there is a general agreement on the close relationship of Cajanus and Atylosia which is separated from each other by Baker (1876) on the basis of strophioled seeds in Atylosia species. Supporting this view, Lackey (1977) considered Cajanus to be a cultigen of Atylosia.

Since the hybridization and the species hybrids have become an integral part of the new systematics, as the findings of such study serve as valuable clues in determining the interrelationship between various species and following their probable mode of evolution. As such, studies on hybridization are essential if related species or taxa are utilized in breeding programme aimed at improvement of cultivated taxon. More so, for developing better plant types the innovative approaches in cytogenetics needs constant reference to the chromosome status and behaviour of the test materials. Many of the desired traits are well distributed in the wild relatives of Cajanus cajan. These are summarised as follows.

| Wild relatives of <u>Cajanus cajan</u> | Desired traits | Author |
|---|----------------------|--------------------|
| <u>Atylosia scarabaeoides</u> | Ped borer resistance | Reddy et al.(1979) |
| <u>Atylosia albicans</u> | High protein content | Reddy et al.(1979) |
| <u>Atylosia volubilis</u> | Wilt resistance | Remanandan (1980) |
| <u>Atylosia lineata</u> | Wilt resistance | Remanandan (1980) |

| | | |
|----------------------------|-------------------|--------------------------------|
| <u>Atylosia platycarpa</u> | Photoinsensitive | Ariyanayagam and Spence (1978) |
| | Blight resistance | Remanandan (1980) |
| <u>Atylosia mollis</u> | Blight resistance | Remanandan (1980) |

Crossability of some Atylosia species with Cajanus cajan has already been demonstrated (Deodikar and Thakar, 1956; Kumar and Thombre, 1958; Kumar *et al.*, 1958; Reddy, 1973; De, 1974; Ariyanayagam and Spence, 1978; Reddy *et al.*, 1980; Reddy and De, 1983; Tripathi *et al.*, 1984, Dundas, 1985; Pundir and Singh, 1985, a,b,c, Kumar *et al.*, 1985; Tripathi and Patil, 1986, Tripathi, 1986 and Yadav, 1986). Interspecific hybrids between Atylosia species has been successfully obtained (Tripathi and Patil, 1984; Pundir and Singh, 1985). Tripathi and Patil (1986) successfully raised trispecific hybrids with genus Atylosia involving Cajanus cajan as seed parent and F_1 of Atylosia cajanifolia x Atylosia scarabaeoides. Furthermore, trispecific hybrid in the subtribe cajaninae involving Cajanus as a seed parent and F_1 of A. cajanifolia x A. scarabaeoides as a pollen parent was successfully produced by Tripathi (1986).

Study of polyploids offers scope to increase the chromatin content of a cell and thus bring about quantitative changes in the gene content which in turn may favourably effect the desirable characters in breeding material. Also, polyploidy has played a great role in the evolution of economic plants. Inspite of reduced fertility, tetraploids are of more economic importance providing gigas vegetative parts. Colchicine has been widely used for inducing polyploidy in different plant materials, but the studies on Cajanus and Atylosia are very few. (Kumar *et al.*(1945); Pathak (1948); Bhattacharjii (1956) in Cajanus cajan and in Atylosia scarabaeoides by Jha (1986).

Mutagenesis has been used to improve morphological as well as physiological characters. The possibility offered by induced mutations to increase variability is of extreme interest to the plant breeder. Every mutation even wheather small or big has great significance for a morphological or

physiological character, as it modifies the naturally established balance in selection of adapted block of genes and thus open avenues for both natural and man made selection. Mutation due to chromosomal changes is of considerable importance in genetic studies. However the informations on the genetic variability induced in Cajanus cajan is meagre (Khan et al., 1973; Khan and Veeraswamy, 1974; Venkateswarly, 1973; Venkateswarlu et al., 1980).

In the light of the above, the present investigation entitled, "Cytogenetical studies in some species of genus Atylosia and Cajanus cajan" was undertaken with the following objectives.

1. To study the external morphology of various species in order to find out diagnostic morphological features between them.
2. To study the karyomorphological features of different species and cultivars with a view to bring out similarities and/or differences amongst the karyotypes of each one of them.
3. To attempt large number of crosses between different species of Atylosia and also with Cajanus cajan, with a view to find out crossability between them and determine the percentage success of crossability in different hybrids.
4. To study in detail the morphological characters of hybrids with respect to dominant recessive relationship.
5. To study the microsporogenesis, particularly the nature of chromosome pairing at diakinesis and metaphase-I, in all the hybrids and to determine the structural homology or differences contributed by the parental species and to find out per cent fertility/sterility of the hybrids.

6. To standardise technique for colchicine treatment, for successful induction of polyploidy, involving all the Atylosia spp., and Cajanus cajan under study.
7. To study the effects of induced polyploidy on morphological features, fertility and chromosome behaviour in C_0 and C_1 generation.
8. To study the effect of Ethyl Methane Sulfonate (EMS) on morphology, fertility and chromosomal behaviour of different Atylosia species and Cajanus cajan (L.) Millsp.

MATERIALS AND METHODS

Materials

Details of experimental materials used in the present study are given in Table-1.

Table-1: EXPERIMENTAL MATERIALS

| Sl. No. | Species | Cultivars/collection | Source |
|---------|--|----------------------|-----------------------|
| 1. | <u>A. albicans</u> (W.&A.) Benth | JM 2337 | ICRISAT, Hyderabad |
| 2. | <u>A. lineata</u> (W.&A.) | JM 3366 JM 2639 | ICRISAT, Hyderabad |
| 3. | <u>A. mollis</u> (Benth.) | JM 2943 | ICRISAT, Hyderabad |
| 4. | <u>A. caianifolia</u> Haines | JM 2739 | ICRISAT, Hyderabad |
| 5. | <u>A. platycarpa</u> Benth. | JM 2873 | ICRISAT, Hyderabad |
| 6. | <u>A. scarabaeoides</u> (L.) Benth. | RJW Collection | ICRISAT, Hyderabad |
| 7. | <u>A. volubilis</u> (Blanco) Gamble | JM 1984 | ICRISAT, Hyderabad |
| 8. | <u>Cajanus cajan</u> (L.) Millsp. | SNT Collection | IGFRI, Jhansi |
| 9. | <u>Cajanus cajan</u> (L.) Millsp. | ICP 8647 | ICRISAT, Hyderabad |

Methods:

Seed germination:

Dry seeds of Cajanus cajan were kept on wet filter paper in petridishes for germination. Germinated seeds were

transferred to earthen pots as well as microplots. Seeds of Atylosia scarabaeoides, Atylosia volubilis, Atylosia albicans, Atylosia lineata and Atylosia cajanifolia were germinated on wet filter paper in petridishes after pretreatment of hot water. For maximum germination in Atylosia mollis and Atylosia platycarpa, seeds were scarified with conc. H_2SO_4 for 30 minutes, and then washed thoroughly and kept on wet filter paper in petridishes. The germinated seeds were transferred to the earthen pots as well as microplots.

Recording of observations

a) Seed germination:

Emergence of radicle was considered as germination of seeds.

b) Morphology

1. Plant height: The height was measured in cm from the ground level to the terminal end of the main flowering shoot.
2. Plant spread: The plant spread was measured in cm across the width of plant.
3. Leaf length: The length of central leaflet was measured in cm from the base of the tip of leaflet.
4. Leaf breadth: The breadth of central leaflet was measured in cm at the widest portion of leaflet.
5. Days to bud initiation: The number of days taken from the date of sowing to the emergence of first developed bud on the main shoot.
6. Days to 50% flowering: The number of days taken from the date of sowing to the date on which 50% of the branches on an individual plant flowered.

7. Days from bud to flower: The number of days taken from the time of bud initiation to its full development into flower.
8. Days from pod initiation to maturity: The number of days taken from the date of first visibility of pod emergence to its maturity.
9. Days to 50% maturity: The number of days taken from the date of sowing to the date on which 50% of all the pods on a plant matured.
10. Pod setting: Pod setting was determined on the number of full matured pods per 100 buds.

$$\text{Pod set \%} = \frac{\text{Number of pods formed}}{\text{No. of buds studies}} \times 100$$
11. Ovule fertility: Ovule fertility was determined on the number of fully formed seeds per 100 ovules.

$$\text{Ovule fertility \%} = \frac{\text{Total No. of seeds}}{\text{Total No. of chambers}} \times 100$$
12. Length of pod: Measured in cm from the base of the pod to tip of its beak.
13. Breadth of pod: Measured in cm from the middle of the pod.
14. Pollen fertility: Pollen fertility was estimated by stainability of pollen grains in acetocarmine. Those pollen grains which stained brightly were taken as fertile and those which remained unstained were recorded as sterile. Pollen size was measured using 15x eye piece and 40x objective. Fifty different microscopic fields using 15x eye piece and 10x objective lense were scanned and the average values expressed as percentage of fertile pollen. The observed diameter of pollen grains were multiplied by the correction factor (3.0) to get the actual size and the average were calculated.

Cytological techniques

Mitosis:

The mitotic studies were made only from young growing root tips obtained from germinated seeds. The proper time of collection of root tips for somatic metaphase was found to be between 10.30 and 11.30 a.m. during the summer.

Fixation:

Root tips were thoroughly washed in water and then fixed in 1:3 propiono-alcohol to which a traces of ferricchloride was added to increase the stainability. Fixation for 24 hours was necessary for better staining.

Staining:

Fixed root tips were stained in 1% propionocarmine for 15-30 minutes. The deeply stained portion of the root tips was cut and squashed in 1% propionocarmine. The chromosomes were separated by repeated heating, mild tapping and a little pressing. The prepared slides were sealed with parafin wax for detailed study.

Karyotypic studies:

For Karyotypic studies in different species of Atylosia, cultivar/collection of Cajanus cajan their hybrids and tetraploides, the chromosomes were arranged in linear order, according to their total length and measured in mm and converted in Micron. The chromosomes have been classified into the following types on the basis of their total length.

| <u>Chromosome type</u> | | <u>Length of chromosome</u> |
|------------------------|--------|-----------------------------|
| A | Long | 3.00 - 4.26 μ |
| B | Medium | 2.00 - 2.99 μ |
| C | Short | 1.41 - 1.99 μ |

T.F. value was calculated with the help of the following formula (Huziwara, 1962).

$$\text{T.F. value} = \frac{\text{Sum of short arm length}}{\text{sum of total chromosome length}} \times 100$$

Meiosis:

Meiotic studies were made in pollen mother cells (abbreviated hereafter in their text as PMC) from young flower buds of suitable size. The most suitable time for collection of flower buds was found to be between 8.30 a.m. to 9.30 a.m. The flower buds were fixed in 1:2 propiono alcohol or in Carnoy's fluid (6:3:1 - absolute alcohol 6 part: chloroform 3 part : propionic acid 1 part). Fixed flower buds were kept overnight and smeared in 1% propionocarmine. All the stages of meiosis right from prophase-I to the pollen formation were analysed.

The cytological analysis were made from temporary slides and suitable cells were photographed on 35 mm film with Olympus PM-6 microphotographic camera at 100 x 15 and 100 x 10 magnifications.

Crossability studies:

Both interspecific and intergeneric crosses were attempted reciprocally for studying the crossability relationship. Suitable flower buds were emasculated. Anthers were removed from the flower buds with the help of a forcep, by opening their keels from one end. In the morning hours following emasculation, the pollen grains were taken from freshly opened flowers of desired male parent and gently applied to the stigmas of emasculated flowers of the female parent. After pollination the flowers were covered with butter paper bags. The pollinated flowers were properly labelled. The flower bud size of one day before flower opening was most suitable for pollination. The seeds collected from the possibly crossed pods and those of the parents were germinated on wet filter paper in petridishes and the germinated seeds were transferred to earthen pots/microplots. The F_1 plants were detected by comparing them with their respective parents.

*Dr. R. S. ...
22-11-57
10/11/57*

The percentage success of each cross was calculated as follows:

- a)
$$\text{Number of crossed pods} = \frac{\text{No. of } F_1 \text{ plants}}{\text{No. of total plants}} \times \text{No. of possibly crossed pod harvested}$$
- b)
$$\text{Crossability percent} = \frac{\text{No. of crossed pod}}{\text{No. of pollinated flowers}} \times 100$$

In the cases where hybridization failed to occur, pistils were collected and fixed for 24 hours in F.P.A. (Formalin 5 : propionic acid 5 : 70% alcohol 90 v/v), 4-6 hours after pollination. The tissue were cleared in luctophenol and kept in lactophenol for 16 hours, mounted on glass slides in cotton blue and observed under a microscope for germination of the pollen grains.

Induction of polyploidy

For induction of polyploidy, the following methods are used.

1) Seed treatment:

Selected healthy seeds were soaked in water for 16 hours so as to initiate cell division before colchicine treatment. Soaked seeds of Atylosia species and Cajanus cajan were treated with 0.05%, 0.1% and 0.2% aqueous colchicine solutions for 2-24 hours. Ten seeds were used for each treatment. The treated seeds were washed well and placed on wet filter paper to see their germination. Germinated seeds transferred to the earthen pots for seedling emergence.

11) Seedling treatment

a) Immersion method:

The seedlings of Atylosia species and Cajanus cajan were raised in petridishes and 4-6 days old seedlings were inverted in 0.05%, 0.1% and 0.2% aqueous colchicine solutions,

in a shallow container for 2-8 hours. Roots were kept outside and moist with cotton plugs soaked with water. After each treatment, the seedlings were thoroughly washed and transferred to the earthen pots.

b) Colchicine treatment through cotton plug method:

Seedlings of Alyosia species and Cajanus cajan were raised in the plastic pots containing field soil during the month of July. When the first pair of leaves opened out fully (after 8-10 days of sowing), the apical buds were treated with 0.05%, 0.1% and 0.2% aqueous colchicine solutions for 8 hours a day for 1-3 days. Absorbent cotton plug were kept on the apical bud and moistened with different concentrations of aqueous colchicine solutions 8 hours a day for 1 to 3 days. The treatment was carried out from 9 a.m. to 5 p.m. After the respective treatments, the shoot apex of each seedling was thoroughly washed with water.

Criteria used for judging polyploidy

All seedlings specially those which showed morphological alterations such as dark green pigmentation, thicker and coarser leaves were screened for further studies. The stomata size and frequency per unit area were the criteria used in initial screening. Later on, pollen size and number were studied. The final confirmation of induced polyploidy was based on chromosome counts in pollen mother cells.

a) Stomata size and number:

A thin layer of lower surface of the leaf of young seedling was taken off and used for studying the size and number of stomata. Stomata per unit area were recorded by using 15x eye piece and 40 x objective lenses. The size was measured on 20 stomata under 15x X 40x power and the observed value were multiplied by correction factor (3.0) to determine the actual size.

b) Pollen fertility, size and number:

Observations on these parameters were recorded as per the procedure described previously.

Induction of mutation:

For inducing mutations EMS (Ethyl Methane Sulfonate- $\text{CH}_3\text{SO}_3\text{C}_2\text{HS}$) was used as a chemical mutagen. For inducing mutations in Alylosia spp. and Cajanus cajan (ICP 8647 and SNT Coll.) EMS solution of different concentrations were used. For each treatment 50 healthy seeds were soaked in water for 16 hours and soon after dipped in 0.2%, 0.4%, 0.6%, 0.8% and 1.0% freshly prepared aqueous solutions of EMS for 4 and 8 hours, at room temperature.

After each treatment, the seeds were washed thoroughly with water to ensure complete removal of accumulated chemical mutagen. The EMS treated seeds were placed on wet filter paper in petridishes to see their germination. The germinated seeds were finally transferred to micro plots for the emergence of seedlings in the field.

Morphology of Atylosia species and Cajanus cajan

Atylosia platycarpa: (JM2873)

A herbacious twiner (Plate-1; Fig-4), branches very slender, climbing, densely clothed with short spreading grey hairs. Petiole: 2.8-4.0 cm, stipules minute, linear, persistent. Leaflets: round cuspidate, 3.0-3.0 cm long and 3.0-4.8 cm broad, greenish on both surfaces, Petiolule: 1.0-1.5 cm, Peduncles: Shorter than the petioles, suppressed the end of the shoots, where the leaves also are much reduced. Pedicel: 0.8-1.0 cm, as long as the calyx. Calyx: 0.8-1.0 cm, densely hairy, teeth linear setaceous. Pod: green flat, 2.5-4.0 cm long and 1.0 cm broad, distinctly lineate, clothed with short deciduous spreading hairs.

Atylosia mollis: (J.M. 2943)

A herbacious twiner (Plate-1; Fig.-5) branches firm slender, Petiole: 1.5-2.5 cm, leaflets: coriaceous, green, obovate, spathulately narrowed to a rounded base. Racemes: 5.0-7.0 cm long, loose, short peduncled. Pedicel: 0.8-1.0 cm in length, Bracteoles: large, roundish, forming a tuft before the racemes expand. Calyx: lanceolate, 0.3-0.5 cm long. Corolla: Yellow, standard, 1.5-1.6 cm in length, Pod: Straight, 3.0-4.0 cm in length, 1 cm in breadth, green, 2-5 seeded, rounded at both ends.

Atylosia lineata (JM 3366)

An erect shrub (Plate-1; Fig.7) with long straight grooved branches, Stipules minute, hairy, Petiole 1.5 - 3.0 cm in length, Leaflets: Sub-coriaceous, green, hairy, lanceolate, triplinerved, Flower: axillary, Calyx: 0.5-0.8 cm in length, teeth deltoid, the lowest one is longer, Corolla: 1.4-1.5 cm long, yellow, persistent, Pod: green, straight, 2.0-3.5 cm long, 0.7-0.9 cm broad, densely covered with fine spreading hairs, Seeds: light brown with dark brown dots.

Atylosia lineata (JM 2639)

An erect shrub (Plate-1; Fig.6) with long straight grooved branches. Stipules: minute, hairy, Petiole: 1.6-3.5 cm in length, Leaflets: subcoriaceous, green, hairy, lanceolate, triplinerved, Flowers: racemed, Calyx: 0.5-0.7 cm in length, teeth deltoid, Corolla: 1.5-1.6 cm in length, yellow with purple streaks, persistent, Pod: green, straight, 1.5-2.5 cm in length, 0.4-0.6 cm in width, covered with short spreading hairs. Seeds: brown with black dots.

Atylosia scarabaeoides (RJW Collection)

A herbaceous twiner (Plate-1; Fig-8) biennial, with twining branches. Stipules: Minute, persistent.

Petiole: 1.0-2.0 cm, in length, Leaflets: 2.0-3.0 cm long, 1.0-1.5 cm broad, obovate, triplenerved in the lower half. Peduncle: Short, 2-6 flowered, Pedicel: 0.4-0.6 cm in length, Calyx: 0.4-0.6 cm in length, teeth linear, lowest one is longer, Corolla: yellow with red stripes, 0.6-0.8 cm in length, Pod: green, straight, distinctly lineate, 1.5-2.5 cm in length, 0.5 cm in width, covered with fine spreading silky hairs.

Atylosia cajanifolia (JM 2739)

An erect shrub (Plate-1; Fig.1) with long straight grooved branches. Stipules: minute, hairy; Petiole: 1.5-2.0 cm in length, Leaflets: subcoriaceous, green, hairs thin, lanceolate, triplinerved, palmately reticulate, Calyx: 0.5-0.7 cm in length, teeth deltoid, Corolla: 1.5-1.6 cm in length, complete dark red, Pod: straight, 2.0-4.0 cm in length, 0.5-1.0 cm in breadth, colour of pod- brown, covered with dense spreading hairs.

Atylosia volubilis (JM 1984)

A shrubby twiner (Plate-1; Fig.3) branches slender, grooved, leaflets: cuspidate, leaf apices acute, 3.0-5.0 in length, green, much narrowed in the lower half the leaf base deltoid, Stipules:

minute, persistent, Petiole: 3.0-4.0 cm in length, bracteoles large, roundish, forming a conspicuous tuft before the opening of racemes, Calyx: 0.4-0.6 cm in length, persistent, Corolla: yellow 1.6-1.7 cm in length, Pod: green straight, 2.0-3.5 cm in length and 1.0 cm in breadth, narrowed to the base, beaked.

Atylosia albicans (JM 2337)

A shrubby twiner (Plate-1; Fig-2) branches slender, grooved, leaflets obovate, leaf apices oval, 3.0-4.8 cm in length, green, much narrowed in the lower half, the leaf base little rounded. Stipules: minute, persistent, Petiole: 3.0-4.5 cm long, Racemes: 1-12 flowered, usually shorter than the leaves, bracts small, round persistent, pedicel 0.7-1.0 cm long, calyx: 0.5-0.6 cm in length, lowest tooth lanceolate, corolla: 1.5-1.6 cm long, yellow, pod: 1.5-2.6 cm long, 0.6-0.8 cm broad, green straight, distinctly lineate, narrowed to the base and beaked.

Cajanus cajan (SNT Collection)

An erect shrub (Plate-1; Fig.9) branches grooved, stipules minute, lanceolate, fugacious, Leaflets: green, non-hairy, oval-oblong with emarginate leaf apices, 3.0-5.0 cm in length, 1.0-2.5 cm in width, Flowers: in sparse, distinctly peduncled, Pedicel: 1.0-1.5 cm long, Calyx: 0.4-0.6 cm in length, Corolla: yellow, 1.5-1.6 cm in length, 1.4-1.5 cm in width, deciduous, Pod: 3.0-5.0 cm in length, 0.5-0.8 cm in width, green with black streaks, non-hairy, non-shattering, beak prominent. Seed: Yellowish brown in colour, non-strophioled.

Cajanus cajan (ICP 8647)

Erect shrub (Plate-1; Fig.10) branches grooved, Stipules: minute, lanceolate, fugacious, Leaflets green, non-hairy, lanceolate. 4.0-6.0 cm in length, 1.0-2.0 cm in

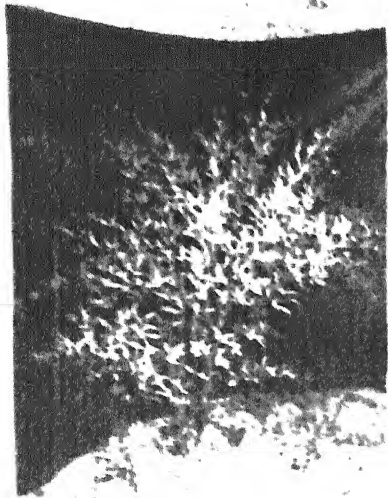
width, Flowers in sparse distinctly peduncled, Pedicel: 1.0-1.5 cm in length, Calyx: 0.6 cm long, Corolla: Yellow, 1.4-1.6 cm in length, 1.3-1.5 cm in width, deciduous, Pod: 4.0-6.0 cm in length, 0.5-0.9 cm in width, non-hairy, green with black streaks, non-shattering, beak prominent. Seed: light brown, non strophioled.

Detailed morphological observations in different species of Atylosia and two cultivars of Cajanus cajan are summarised in Table-2.

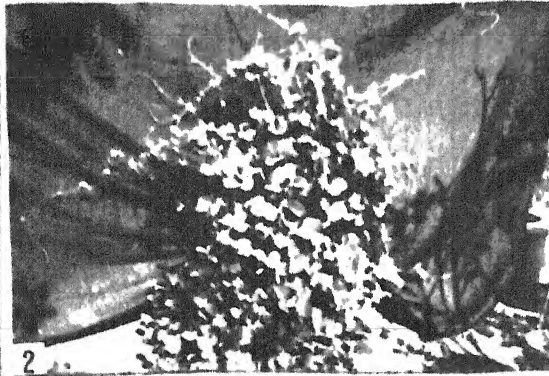
PLATE - 1.

- Fig. 1. Atylosia cajanifolia
Fig. 2. Atylosia albicans
Fig. 3. Atylosia volubilis
Fig. 4. Atylosia platycarpa
Fig. 5. Atylosia mollis.
Fig. 6. Atylosia lineata (JM 2639)
Fig. 7. Atylosia lineata (JM 3366)
Fig. 8. Atylosia scarabaeoides
Fig. 9. Cajanus cajan (SNT Coll.)
Fig. 10. Cajanus cajan (ICP 8647)

PLATE - 1



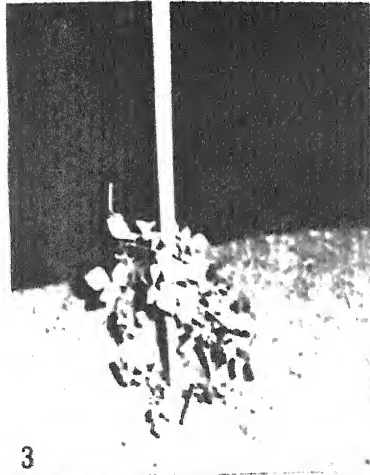
1



2



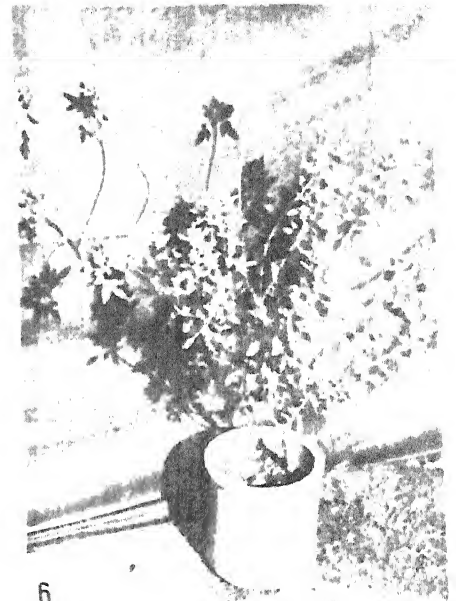
4



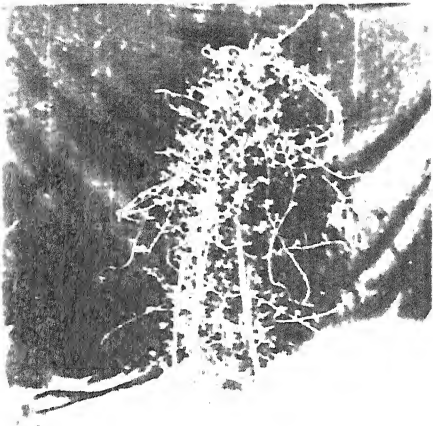
3



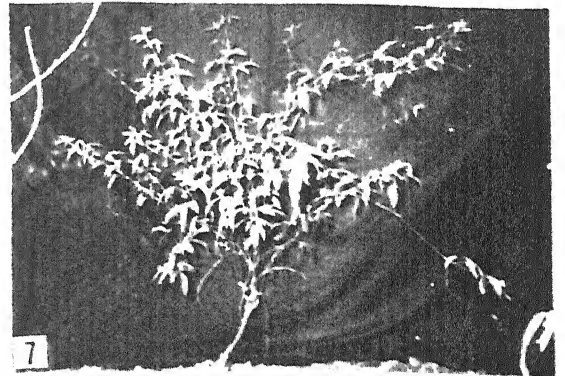
9



6



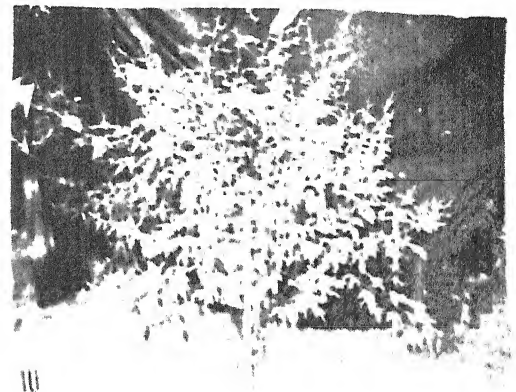
5



7



8



10

Table - 2

Morphological observations on different species of *Atylosia* and 2 cultivars of *Cajanus cajan* (av. of 5 plants)

| CHARACTERS | <i>A. calanifolia</i> | <i>A. albicans</i> | <i>A. volubilis</i> | <i>A. platycarpa</i> | <i>A. mollis</i> | <i>A. lineata</i> (JM 2639) | <i>A. lineata</i> (JM 3366) | <i>A. scarabaeoides</i> | <i>C. cajan</i> (SMT coll.) | <i>C. cajan</i> (ICP 8647) |
|---|-----------------------|----------------------|----------------------------|----------------------------------|-------------------------------|--------------------------------|----------------------------------|-------------------------|--------------------------------|-------------------------------|
| Germination | Hypogeal | Hypogeal | Hypogeal | Hypogeal | Hypogeal | Hypogeal | Hypogeal | Hypogeal | Hypogeal | Hypogeal |
| Shape of first pair of simple leaves | Lanceolate | Ovate | Ovate | Lanceolate | Ovate | Ovate | Ovate | Ovate | Lanceolate | Lanceolate |
| Growth habit | Erect shrub | Twining shrub | Herbaceous creeper | Twining herb | Erect shrub | Erect shrub | Herbaceous creeper | Erect shrub | Erect shrub | Erect shrub |
| Branching | Acute angled | Acute angled | Acute angled | Acute angled | Acute angled | Nearly right angled | Nearly right angled | Acute angled | Acute angled | Acute angled |
| No. of pr. branches | 4.0 | 13.3 | 8.5 | 4.5 | 6.11 | 4.50 | 7.0 | 6.5 | 7.1 | 6.2 |
| No. of sec. branches | 6.21 | 15.5 | 12.0 | 6.5 | 10.50 | 8.61 | 11.13 | 10.5 | 16.8 | 17.3 |
| Central leaflet: | | | | | | | | | | |
| shape | Lanceolate | Obovate | Cuspidate | Cuspidate | Obovate | Lanceolate | Lanceolate | Obovate | Oval-oblong | Lanceolate |
| surface | Hairy | Non-hairy | Non-hairy | Hairy | Hairy | Hairy | Hairy | Hairy | Non-hairy | Non-hairy |
| (L x B) cm. | 5.0x2.10 | 4.3x 3.1 | 4.1x4.0 | 4.5x4.0 | 2.8x2.40 | 5.0x2.0 | 4.9x2.1 | 2.65x1.35 | 4.6x1.8 | 5.5x1.6 |
| venation | Palmately reticular | Palm. retic. | Palm. retic. | Palm. retic. | Palm. retic. | Palm. retic. | Palm. retic. | Palm. retic. | Palm. retic. | Palm. retic. |
| Length of petiole (cm.) | 1.70 | 4.00 | 3.6 | 3.0 | 1.80 | 2.41 | 1.91 | 1.56 | 2.50 | 2.6 |
| Leaf apex | Acute | Oval | Acute | Acute | Acute | Acute | Acute | Acute | Emergent | Acute |
| Nature of stipules | Persistent | Persistent | Persistent | Persistent | Persistent | Persistent | Persistent | Persistent | Pugacious | Pugacious |
| Stem: | | | | | | | | | | |
| colour | Green | Green | Green | Green | Green | Green | Green | Green | Green | Green |
| woody/soft | Soft | Soft | Soft | Soft | Soft | Soft | Soft | Soft | Soft | Soft |
| Days from sowing to bud initiation | 108 | 120 | 181 | 51 | 80 | 110 | 112 | 90 | 93 | 130 |
| Days from sowing to 50% flowering | 121 | 134 | 205 | 60 | 90 | 128 | 124 | 99 | 107 | 145 |
| Days between bud to flower | 12 | 11 | 15 | 7 | 9 | 13 | 11 | 9 | 13 | 15 |
| Days between pod initiation to maturity | 38 | 33 | 38 | 27 | 37 | 37 | 35 | 30 | 35 | 37 |
| Flower: | | | | | | | | | | |
| size of the standard | 1.6x1.6 | 1.6x1.5 | 1.7x1.6 | 1.1x0.9 | 1.6x1.6 | 1.5x1.4 | 1.5x1.3 | 0.71x0.55 | 1.6x1.5 | 1.5x1.4 |
| petal (L x B) cm. | | | | | | | | | | |
| colour of the standard | Red | Brownish yellow | Yellow | Yellow | Yellow | Yellow with purple streaks | Yellow | Yellow with red stripes | Yellow | Yellow |
| red petal | | | | | | | | | | |
| Nature of petals | Persistent | Persistent | Persistent | Persistent | Persistent | Persistent | Persistent | Persistent | Deciduous | Deciduous |
| Length of style (cm.) | 1.5 | 1.5 | 1.6 | 1.2 | 1.6 | 1.5 | 1.4 | 0.70 | 1.6 | 1.5 |
| Pod: | | | | | | | | | | |
| colour of pod | Brown | Green | Green | Green | Green | Green | Green | Green | Green with black streaks | Green with black streaks |
| (L x B) cm. | 3.8x0.56 | 2.0x0.7 | 2.5x1.0 | 3.6x1.0 | 3.4x1.0 | 1.5x0.42 | 2.2x0.60 | 1.9x0.50 | 4.0x0.7 | 4.9x0.7 |
| hairs on mature pod | Present | Absent | Absent | Present | Absent | Present | Present | Present | Absent | Absent |
| beak of pod | Prominent | Prominent | Prominent | Prominent | Prominent | Minute | Minute | Minute | Prominent | Prominent |
| thickness of pod | 0.533 | 0.36 | 0.504 | 0.308 | 0.509 | 0.40 | 0.28 | 0.306 | 0.70 | 0.73 |
| nature of mature pod | Shattering | Shattering | Shattering | Shattering | Shattering | Shattering | Shattering | Shattering | Non-shatt. | Non-shatt. |
| Seed: | | | | | | | | | | |
| colour | Red | Grey with black dots | Dark brown with black dots | Light brown with dark brown dots | Reddish brown with black dots | Brown with black dots | Light brown with dark brown dots | Brown with black dots | Dark brown | Light brown |
| thickness of seed (cm.) | 0.400 | 0.28 | 0.20 | 0.300 | 0.40 | 0.35 | 0.28 | 0.20 | 0.48 | 0.43 |
| No. of chambers per pod | 2.92 | 2.61 | 3.10 | 2.7 | 2.51 | 1.81 | 1.66 | 3.2 | 3.2 | 3.2 |
| No. of seeds per pod | 2.80 | 2.4 | 2.5 | 2.10 | 1.60 | 1.50 | 2.5 | 2.5 | 2.4 | 3.0 |
| Strophils | Present | Present | Present | Present | Present | Present | Present | Present | Absent | Absent |
| Days to 50% maturity | 195 | 225 | 227 | 128 | 155 | 195 | 180 | 151 | 185 | 210 |
| Pod set (%) | 36.66 | 61.50 | 50.0 | 74.0 | 40.0 | 62.0 | 60.0 | 62.7 | 28.9 | 27.5 |
| Ovule fertility (%) | 91.0 | 71.2 | 67.2 | 95.5 | 61.5 | 83.0 | 85.0 | 88.0 | 85.0 | 81.2 |
| Stomata: | | | | | | | | | | |
| frequency | 6.8 | 8.50 | 8.0 | 8.5 | 8.0 | 7.0 | 6.5 | 6.8 | 6.0 | 6.0 |
| (L x B) μ | 18.0x15.0 | 12x9 | 15x12 | 12.0x9.0 | 15.0x12.0 | 15.3x12.1 | 12.0x9.0 | 12.0x9.0 | 15.0x12.0 | 14.0x12.0 |
| Spread of plant (cm.) | 125.8 | 85.31 | 78.0 | 32.51 | 45.0 | 105 | 115 | 35.0 | 177.0 | 170.0 |

CYTOLOGY OF ATYLOSIA SPECIES AND CAJANUS CAJAN:

Atylosia mollis (JM 2943)

Mitosis

At somatic metaphase 22 chromosomes were observed (Fig. 5). The chromosome complement of Atylosia mollis comprised A, B and C classes (Table 3). A includes 3 pairs of chromosomes, having length between 3.05 μ to 3.54 μ . Two of which have submedian, one possesses median primary constriction. The longest chromosome of class A possesses secondary constriction in its short arm. Length of satellite was 0.35 μ . Class B includes 6 pairs of chromosomes, all having length between 2.12 to 2.84 μ . Of these, two pairs of chromosomes have median and two pairs of chromosomes have submedian primary constriction and the rest two have subterminal primary constriction. The class C includes two pairs of chromosomes 1.77 μ in length. Both of these have submedian primary constriction.

Thus total chromosome length varied from 1.77 to 3.54 μ . Length of total chromosome complement was 56.76 μ and T.F. % 42.98 (Table-3).

Meiosis

Meiotic study revealed eleven bivalents at diakinesis and metaphase-I (Plate-2; Fig.6) regularly. Ring and rod bivalents at metaphase-I ranged from 9-11 and 0-2 with 10.42 and 0.58 per cell respectively (Table-5). Chiasma frequency as revealed by diakinesis was 21.42 per cell and 1.94 per bivalent (Table-4). At anaphase-I and II, regular separation of 11-11 chromosomes (Plate-2; Fig-7) to the poles was observed. At sporad stage regular tetrad formation was observed which resulted in high pollen fertility (99.6%). Fertile pollen size ranged from 33 to 36 μ with 34.5 μ mean diameter.

Table - 3

Observations on somatic chromosome complement of Atylosia mollis.

| Pair No. | Class | Position of Constriction | | Length of short arm (μ) | Length of long arm (μ) | Total chromosome length μ | L/S arm ratio |
|----------|-------|--------------------------|----------------|-------------------------------|------------------------------|-------------------------------|---------------|
| | | Pri- mary | Sec- ondary | | | | |
| 1 | A | SM | SAT | 1.42+0.35 | 1.77 | 3.54 | 1.00 |
| 2 | A | M | | 1.63 | 1.63 | 3.36 | 1.00 |
| 3 | A | SM | | 1.42 | 1.63 | 3.05 | 1.14 |
| 4 | B | M | | 1.42 | 1.42 | 2.84 | 1.00 |
| 5 | B | SM | | 1.27 | 1.57 | 2.84 | 1.22 |
| 6 | B | ST | | 0.71 | 2.13 | 2.84 | 3.00 |
| 7 | B | ST | | 0.71 | 1.42 | 2.13 | 2.00 |
| 8 | B | M | | 1.06 | 1.06 | 2.12 | 1.00 |
| 9 | B | SM | | 1.00 | 1.12 | 2.12 | 1.12 |
| 10 | C | SM | | 0.71 | 1.06 | 1.77 | 1.49 |
| 11 | C | SM | | 0.71 | 1.06 | 1.77 | 1.49 |

$$\text{T.F. \%} = \frac{24.4}{56.76} \times 100 = 42.98\%$$

Karyotypic Formula:-

$$1 \text{ A (M)} + 2 \text{ A (SM)} + 2 \text{ B (M)} + 2 \text{ B (SM)} + 2 \text{ B (ST)} + 2 \text{ C (SM)}$$

Table - 4

Chiasma frequency in Atylosia mollis

| Stage | No. of cells studied | Rivalents with | | Total Xmeta | Xmeta per cell | Xmeta per bivalent |
|------------|----------------------|----------------|-------|-------------|----------------|--------------------|
| | | 2 Xmeta | 1 Xma | | | |
| Diakinesis | 50 | 521 | 29 | 1071 | 21.42 | 1.94 |

Table - 5

Chromosome association at Metaphase - 1 in Atylosia mollis

| No. of cells studied | Chromosome association at M - 1 | | No. of cells per each type | Frequency per cent | Pollen fertility % |
|----------------------|---------------------------------|--------|----------------------------|--------------------|--------------------|
| | Ring II | Rod II | | | |
| 50 | 11 | - | 30 | 60.0 | 99.6 |
| | 10 | 1 | 11 | 22.0 | |
| | 9 | 2 | 9 | 18.0 | |
| Range | 9 - 11 | 0 - 2 | | | |
| Mean | 10.42 | 0.58 | | | |

Atylosia volubilis (JM 1984)Mitosis

Somatic metaphase revealed 22 chromosomes (Plate-2; Fig.1). The chromosome complement of Atylosia volubilis comprised two classes i.e. A and B. Class. A includes three pairs of chromosomes, all having length between 3.55 to 3.90 μ . One of which has secondary constriction in its short arm along with submedian primary constriction. Length of satellite was 0.35 μ . The other two pairs have submedian primary constrictions. Class B includes 8 pairs of chromosomes, having length between 2.13 to 2.84 μ , out of three, two pairs of chromosomes with median, four with submedian and two with subterminal primary constrictions were recorded. In class B₂ among four submedian chromosomes, one has secondary constriction with a satellite, 0.35 μ in length.

Thus, the total chromosome length ranged from 2.13 to 3.90 μ . Length of total chromosome complement of A. volubilis was 63.14 μ and I.F. percentage as 40.63 (Table-6).

Meiosis

Meiotic study revealed eleven bivalents at diakinesis and metaphase-I (Plate-2, Fig; 2,3). At diakinesis two pairs of chromosomes were seen attached to the nucleolus which reflects the presence of two SAT chromosome pairs. At metaphase-I ring and rod Bivalents ranged from 8-11 and 0-3 with 10.16 and 0.84 per cell respectively (Table-8). Chiasma frequency was 21.05 per cell and 1.9 per bivalent (Table-7). At anaphase-I and II equal separation of chromosomes (Plate-2; Fig. 4) was recorded. At sporad stage regular tetrad formation was noticed.

Pollen fertility was 99.6 per cent and fertile pollen size ranged from 30 to 36 μ with 33.5 μ mean diameter.

Table - 6

Observations on somatic chromosome complement of Atylosia volubilis.

| Pair No. | Class | Position of constriction | | Length of short arm in (μ) | Length of long arm in (μ) | Total chromosome length (μ) | L/S arm ratio |
|----------|-------|--------------------------|----------------|----------------------------------|---------------------------------|-----------------------------------|---------------|
| | | Pri- mary | Secun- dary | | | | |
| 1 | A | SM | SAT | 1.42+0.35 | 2.13 | 3.90 | 1.20 |
| 2 | A | SM | | 1.42 | 2.13 | 3.55 | 1.50 |
| 3 | A | SM | | 1.42 | 2.13 | 3.55 | 1.50 |
| 4 | B | M | | 1.42 | 1.42 | 2.84 | 1.00 |
| 5 | B | M | | 1.42 | 1.42 | 2.84 | 1.00 |
| 6 | B | ST | | 0.71 | 2.13 | 2.84 | 3.00 |
| 7 | B | SM | SAT | 1.06+0.35 | 1.52 | 2.83 | 1.07 |
| 8 | B | SM | | 1.06 | 1.42 | 2.48 | 1.33 |
| 9 | B | ST | | 0.71 | 1.77 | 2.48 | 2.49 |
| 10 | B | SM | | 0.92 | 1.20 | 2.13 | 2.00 |
| 11 | B | SM | | 0.92 | 1.20 | 2.13 | 2.00 |

$$\text{T.F. \%} = \frac{25.66}{63.14} \times 100 = 40.63$$

Karyotypic formula:

$$3 \text{ A (SM)} + 2 \text{ B (M)} + 4 \text{ B (SM)} + 2 \text{ B (ST)}.$$

Table - 7

Chiasma frequency in Atylosia volubilis

| Stage | No. of cells studied | Bivalents with | | Total Xmata | Xmata per cell | Xmata per bivalent |
|------------|----------------------|----------------|-------|-------------|----------------|--------------------|
| | | 2 Xmata | 1 Xma | | | |
| diakinesis | 40 | 402 | 38 | 842 | 21.05 | 1.9 |

Table - 8

Chromosome association at Metaphase -1 in Atylosia volubilis

| No. of cells studied | Chromosome association at M - 1 | | No. of cells per each type | Frequency per cent | Pollen fertility % |
|----------------------|---------------------------------|--------|----------------------------|--------------------|--------------------|
| | Ring II | Rod II | | | |
| | 11 | 0 | 30 | 60.0 | |
| 50 | 10 | 1 | 6 | 12.0 | 99.6 |
| | 9 | 2 | 6 | 12.0 | |
| | 8 | 3 | 8 | 16.0 | |
| Range | 3 - 11 | 0 - 3 | | | |
| Mean | 10.16 | 0.84 | | | |

Atylosia Scarabaeoides (RJW Coll.)Mitosis:

Mitosis showed chromosome number $2n = 22$ at somatic metaphase (Plate-2; Fig.8). The chromosome complement of Atylosia scarabaeoides consists of A, B and C classes. A includes two pairs of chromosomes between 3.19 and 3.53 micron length and one, out of the two pairs has submedian primary constriction and secondary constriction in its short arm. Length of satellite was 0.44 μ . The other chromosome pair of class A, possessed submedian primary constriction only. Class B includes 7 pairs of chromosomes having length between 2.12 μ to 2.84 μ , in these two have median, four submedian and one has subterminal primary constriction. Class C includes two pairs of chromosomes, both having 1.91 μ length and submedian primary constriction.

Thus the total length of chromosomes ranged from 1.91 μ to 3.53 μ . Total length of chromosome complement of Atylosia scarabaeoides was 56.4 μ with T.F. 343.40 (Table-9).

Meiosis

Meiotic study revealed eleven bivalents at diakinesis and metaphase-I (Plate-2; Fig. 8). Ring bivalents ranged from 9-11 with 10.44 per cell and rod bivalents ranged from 0-2 with 0.66 per cell (Table-11). Chiasma frequency as recorded from diakinesis was 21.3 per cell and 1.93 per bivalent (Table-10). At anaphase-I and II, regular separation of 11-11 chromosomes was noticed (Plate-2; Fig.9). At sporad stage regular tetrad (Plate-2; Fig. 10) formation was recorded.

Pollen fertility percentage was 99.4 and fertile pollen size ranged from 30.0 μ to 33.0 μ with 31.5 μ mean diameter.

Table - 9

Observations on somatic chromosome complement of Atylosia
scarabaeoides.

| Pair No. | Class | Position of constriction | | Length of short arm in (μ) | Length of long arm in (μ) | Total length (μ) | L/s arm ratio (μ) |
|----------|-------|--------------------------|----------------|----------------------------------|---------------------------------|------------------------|-------------------------|
| | | Pri- mary | Sec- ondary | | | | |
| 1 | A | SM | SAT | 1.27±0.49 | 1.77 | 3.53 | 1.00 |
| 2 | A | SM | | 1.42 | 1.77 | 3.19 | 1.24 |
| 3 | B | SM | | 1.23 | 1.61 | 2.84 | 1.30 |
| 4 | B | SM | | 1.06 | 1.77 | 2.83 | 1.66 |
| 5 | B | SM | | 1.06 | 1.77 | 2.83 | 1.66 |
| 6 | B | M | | 1.06 | 1.06 | 2.12 | 1.00 |
| 7 | B | SM | | 0.92 | 1.20 | 2.12 | 1.08 |
| 8 | B | M | | 1.06 | 1.06 | 2.12 | 1.00 |
| 9 | B | ST | | 0.71 | 1.41 | 2.12 | 2.00 |
| 10 | C | SM | | 0.71 | 1.20 | 1.91 | 1.69 |
| 11 | C | SM | | 0.78 | 1.13 | 1.91 | 1.44 |

$$T.F. \% = \frac{25.00}{56.4} \times 100 = 43.40$$

Karyotypic Formula:

$$2A (SM) + 2B (M) + 4 B (SM) + 1 B (ST) + 2 C (SM)$$

Table - 10

Chiasma frequency in Atylosia scarabaeoides.

| Stage | No. of cells studied | Bivalents with | | Total | Xmata per cell | Xmata per bivalent |
|------------|----------------------|----------------|------|-------|----------------|--------------------|
| | | 2 Xmata | 1Xma | Xmata | | |
| Diakinesis | 50 | 515 | 35 | 1065 | 21.3 | 1.93 |

Table - 11

Chromosome association at Metaphase - 1 in Atylosia scarabaeoides.

| No. of cells studied | Chromosome association at M-1 | | No. of cells per each type | Frequency per cent | Pollen fertility % |
|----------------------|-------------------------------|-----------|----------------------------|--------------------|--------------------|
| | Ring | II Rod II | | | |
| 60 | 11 | 0 | 30 | 49.9 | 99.4 |
| | 10 | 1 | 20 | 32.0 | |
| | 9 | 2 | 10 | 16.6 | |
| Range | 9 - 11 | 0 - 2 | | | |
| Mean | 10.44 | 0.66 | | | |

PLATE - 2

- Fig. 1. Somatic chromosomes of A. volubilis (X 1500)
- Fig. 2. 11 bivalents of A. volubilis at diakinesis - I (X 1500)
- Fig. 3. 11 bivalents of A. volubilis at Metaphase - I (X 1500)
- Fig. 4. Equal separation of 11-11 chromosomes of A. volubilis at Anaphase - I (X 1500)
- Fig. 5. Somatic chromosomes of A. mollis (X 1500)
- Fig. 6. 11 bivalents of A. mollis at diakinesis (X 1500)
- Fig. 7. Equal separation of 11-11 chromosomes of A. mollis (X 1500)
- Fig. 8. Somatic chromosomes of Atylosia scarabaeoides at diakinesis (X 1500)
- Fig. 9. 11 bivalent at Metaphase - I of A. scarabaeoides (X 1500)
- Fig. 10. Equal separation of chromatids at Anaphase-II of A. scarabaeoides. (X 1500)
- Fig. 11. Formation of tetrads at sporad stage (X 400)

PLATE - 2

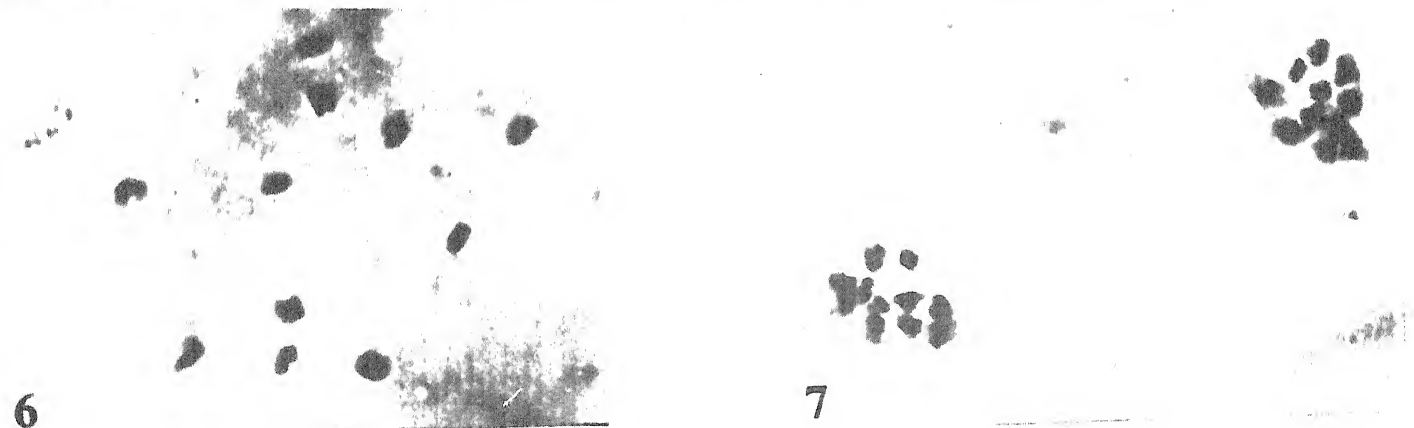
10μ

1 11 11 11 11 11 11 11 11 11 11 11



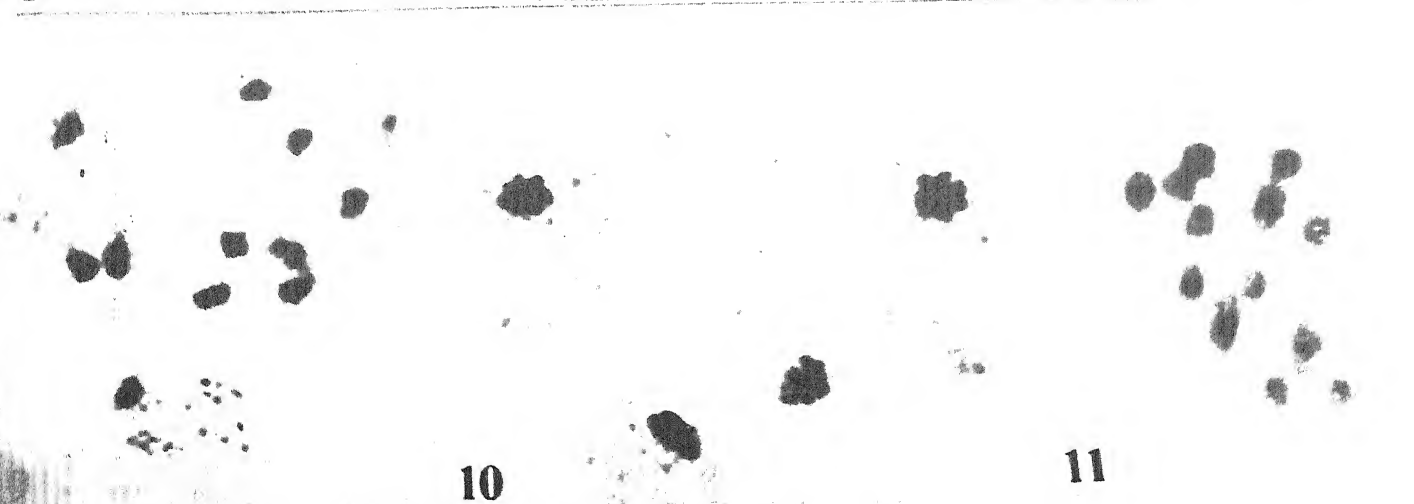
2 3 4

5 11 11 11 11 11 11 11 11 11 11 11



6 7

8 11 11 11 11 11 11 11 11 11 11 11



10

11

Atylosia albicans (JM 2337)Mitosis

Somatic metaphase of root tips cells showed 22 chromosomes regularly (Plate-3; Fig. 1). The chromosome complement of Atylosia albicans consists of two (A & B) classes (Table-12). Class A includes 8 pairs, having length between 3.3 μ to 4.26 microns, two of which have median, four submedian and two subterminal primary constrictions. In these four submedian chromosomes of class A, a long pair or chromosome possesses secondary constriction at subterminal position. Length of satellite was 0.70 μ . Class B includes 3 pairs with length between 2.48 μ to 2.84 μ and with submedian primary constriction. Thus, total chromosome length ranged from 2.48 μ to 4.26 μ . The length of total chromosome complement of A. albicans was 81.6 μ with T.F. % 40.78 (Table 12).

Meiosis

Meiosis showed eleven bivalents at diakinesis and metaphase-I (Plate-3; Fig.2,3). Ring bivalents ranged from 0-7 with 10.34 per cell while rod bivalents ranged from 0-4 with 0.6 bivalents per cell (Table-14). Chiasma frequency as recorded at diakinesis was 21.36 per cell and 1.94 per bivalent (Table-13). At anaphase-I and II, both regular separation of 11-11 chromosomes to the poles (Plate-3; figs.4,5) was noticed resulting in regular tetrad formation and high pollen fertility (99.4%). Fertile pollen size ranged from 33 to 39 μ with 36.0 μ mean diameter.

Atylosia platycarpa (JM 2873)Mitosis

At somatic metaphase 22 chromosomes were observed (Plate-3; Fig.9). It is clear from the table-13. that the

Table - 12

Observations on somatic chromosome complement of Atylosia albicans.

| Chromosome pair No. | Class | Position of constriction | | Length of short arm in (μ) | Length of long arm in (μ) | Total chromosome length μ | L/S arm ratio |
|---------------------|-------|--------------------------|----------------|----------------------------------|---------------------------------|-------------------------------|---------------|
| | | Pri- mary | Sec- ondary | | | | |
| 1 | A | SM | | 1.42+0.71 | 2.13 | 4.26 | 1.00 |
| 2 | A | M | | 2.13 | 2.13 | 4.26 | 1.00 |
| 3 | A | SM | | 1.77 | 2.48 | 4.26 | 1.40 |
| 4 | A | ST | | 1.06 | 3.19 | 4.26 | 3.00 |
| 5 | A | SM | | 1.77 | 2.48 | 4.25 | 1.40 |
| 6 | A | SM | | 1.77 | 2.48 | 4.25 | 1.40 |
| 7 | A | ST | | 1.06 | 2.48 | 3.54 | 2.33 |
| 8 | A | M | | 1.77 | 1.77 | 3.54 | 1.77 |
| 9 | B | SM | | 1.06 | 1.77 | 2.84 | 1.66 |
| 10 | B | SM | | 1.06 | 1.77 | 2.84 | 1.66 |
| 11 | B | SM | | 1.06 | 1.42 | 2.48 | 1.33 |

$$T.F. \% = \frac{33.28}{81.6} \times 100 = 40.78$$

Karyotypic formula:

$$2 A (M) + 4 A (SM) + 2A (ST) + 3 B (SM).$$

Table - 13

Chiasma frequency in Atylosia albicans

| Stage | No. of cells studied | Bivalents with | | Total Xmata | Xmata per cell | Xmata per bivalent |
|------------|----------------------|----------------|-------|-------------|----------------|--------------------|
| | | 2Xmata | 1 Xma | | | |
| Diakinesis | 50 | 518 | 32 | 1068 | 21.36 | 1.94 |

Table - 14

Chromosome association at Metaphase - 1 in Atylosia albicans

| No. of cells studied | Chromosome association at Metaphase - 1 | | No. of cells per each type | Frequency per cent | Pollin fertility % |
|----------------------|---|--------|----------------------------|--------------------|--------------------|
| | Ring II | Rod II | | | |
| 70 | 11 | - | 51 | 71.4 | 99.4 |
| | 10 | 1 | 5 | 7.0 | |
| | 9 | 2 | 8 | 11.2 | |
| | 8 | 3 | 2 | 2.8 | |
| | 7 | 4 | 4 | 5.6 | |
| Range | 0 - 7 | 0-4 | | | |
| Mean | 10.38 | 0.6 | | | |

Table - 15

Observations on somatic chromosome complement of Atylosia
Platycarpa.

| Pair No. | Class | Position of constriction | | Length of short arm in (μ) | Length of long arm in (μ) | Total chromosome length (μ) | L/s arm ratio |
|----------|-------|--------------------------|----------------|----------------------------------|---------------------------------|-----------------------------------|---------------|
| | | Pri- mary | Secun- dary | | | | |
| 1 | A | SM | BAT | 1.42 + 0.35 | 1.77 | 3.55 | 1.00 |
| 2 | B | ST | | 0.71 | 2.13 | 2.84 | 3.00 |
| 3 | B | M | | 1.27 | 1.27 | 2.54 | 1.00 |
| 4 | B | M | | 1.27 | 1.27 | 2.54 | 1.00 |
| 5 | B | SM | | 0.86 | 1.27 | 2.13 | 1.49 |
| 6 | B | ST | | 0.71 | 1.42 | 2.13 | 2.00 |
| 7 | B | SM | | 0.92 | 1.02 | 2.13 | 1.10 |
| 8 | B | SM | | 0.99 | 1.13 | 2.12 | 1.13 |
| 9 | B | SM | | 0.90 | 1.02 | 2.12 | 1.30 |
| 10 | C | SM | | 0.56 | 1.02 | 1.78 | 1.82 |
| 11 | C | M | | 0.85 | 0.85 | 1.70 | 1.00 |

$$\text{T.F. \%} = \frac{20.68}{51.14} \times 100 = 40.43$$

Karyotypic formula:

1 A (SM) + 2 B (M) + 3B (SM) + 2 B (ST) + 16 (SM) + 1C (M)

Table - 16

Chiasma frequency in Atylosia platycarpa

| Stage | No. of cells studied | Bivalents with | | Total Xmata | Xmata per cell | Xmata per bivalent |
|-----------------|----------------------------|----------------|------|----------------|----------------------|-----------------------|
| | | 2 Xmata | lyma | | | |
| Diaki- nasis | 70 | 750 | 20 | 1520 | 21.7 | 1.97 |

Table - 17

Chromosome association at Metaphase -1 in Atylosia platycarpa

| No. of cells studied | Chromosome association at M-1 | | No. of cells per each type | Frequency per cent | Pollen ferti- lity % |
|----------------------------|----------------------------------|--------|-------------------------------------|-----------------------|----------------------------|
| | Ring II | Rod II | | | |
| 85 | 11 | 0 | 69 | 80.7 | 100 |
| | 10 | 1 | 10 | 11.7 | |
| | 9 | 2 | 6 | 7.2 | |
| Range | 9-11 | 0-2 | | | |
| Mean | 10.74 | 0.25 | | | |

chromosome complement of Atylosia platycarpa consists of A, B and C classes. A includes one pair of chromosomes having length 3.55 μ with submedian primary constriction and secondary constriction in its short arm. Length of secondary constriction in its short arm. Length of satellite was 0.35 μ . Class B includes 8 pairs of chromosomes having length between 2.12 μ to 2.84 μ , four pairs have submedian, two with median and two have subterminal primary constriction. Class C includes two pairs having length between 1.70 to 1.78 μ , one pair with submedian and the other pair with median primary constriction. Thus total chromosome length varied from 1.70 to 3.55 μ with the total chromatin length of 41.14 μ and I.F. % 40.43 (Table-15).

Meiosis

Meiosis showed eleven bivalents at diakinesis and metaphase-I (Plate-3; Fig.6). Ring bivalents ranged from 9-11 with 10.74 per cell while rod bivalents ranged from 0-2 with 0.25 per cell (Table-17). Chiasma frequency as revealed by diakinesis was 21.7 per cell and 1.97 per bivalent (Table-16). At anaphase-I and II equal separation of chromosomes to poles (Plate-3; Fig.7) was observed, resulting in formation of four equal daughter nuclei (Plate-3; Fig.8).

Hundred per cent pollen fertility was recorded in A. platycarpa and fertile pollen size ranged from 30 to 36 μ with 33.0 μ mean diameter.

Atylosia cajanifolia (JM 2739)

Mitosis:

Atylosia cajanifolia was observed to have $2n = 22$ chromosomes (Plate-3; Fig.13). The chromosome complement belongs to two classes i.e., A and B (Table-18). Class A includes two

Table - 18

Observations on somatic chromosome complement of Atylosia
cajanifolia.

| Pair No. | Class | Position of constriction | | Length of short arm in (μ) | Length of long arm in (μ) | Total chromo- some length (μ) | L/S arm ratio |
|-------------|-------|-----------------------------|----------------|--|---------------------------------------|---|---------------------|
| | | Pri- mary | Secun- dary | | | | |
| 1 | A | SM | SAT | 1.42+0.35 | 1.77 | 3.54 | 1.00 |
| 2 | A | M | | 1.77 | 1.77 | 3.54 | 1.00 |
| 3 | B | SM | | 1.07 | 1.77 | 2.84 | 1.66 |
| 4 | B | SM | | 1.06 | 1.78 | 2.84 | 1.66 |
| 5 | B | ST | | 0.71 | 2.13 | 2.84 | 3.00 |
| 6 | B | M | | 1.42 | 1.42 | 2.84 | 1.36 |
| 7 | B | SM | | 1.19 | 1.63 | 2.82 | 1.24 |
| 8 | B | M | | 1.41 | 1.41 | 2.82 | 1.00 |
| 9 | B | ST | | 0.71 | 1.91 | 2.62 | 2.69 |
| 10 | B | SM | | 1.19 | 1.42 | 2.61 | 1.48 |
| 11 | B | SM | | 1.06 | 1.42 | 2.48 | 1.33 |

$$\text{T.P. \%} = \frac{27.22}{63.6} \times 100 = 42.78$$

Karyotypic formula:

1 A (M) + 1 A (SM) + 2 B (M) + 5B (SM) + 2B (ST)

Table - 19

Chiasma frequency in Atylosia cajanifolia

| Stage | No. of cells studied | Bivalents with | | Total xmata | xmata per cell | xmata per bivalent |
|------------|----------------------|----------------|-------|-------------|----------------|--------------------|
| | | 2 xmata | 1 xma | | | |
| Diakinesis | 50 | 509 | 41 | 1050 | 21.18 | 1.92 |

Table - 20

Chromosome association at metaphase - 1 in Atylosia cajanifolia

| No. of cells studied | Chromosome association at M-1 | | No. of cells per each type | Frequency per cent | Pollen fertility % |
|----------------------|-------------------------------|--------|----------------------------|--------------------|--------------------|
| | Ring II | Rod II | | | |
| 50 | 11 | 0 | 32 | 64.0 | 99.7 |
| | 10 | 1 | 5 | 10.0 | |
| | 9 | 2 | 5 | 10.0 | |
| | 8 | 3 | 6 | 12.0 | |
| | 7 | 4 | 2 | 4.0 | |
| Range | 7-11 | 0-4 | | | |
| Mean | 10.18 | 0.82 | | | |

PLATE - 3

- Fig. 1. Somatic chromosomes of A. albicans (X 1500)
- Fig. 2. 11 bivalents of A. albicans at diakinesis (X 1500)
- Fig. 3. 11 bivalents of A. albicans at Metaphase-I (X 1500)
- Fig. 4. Equal separation of 11-11 chromosomes of A. albicans at Anaphase-I (X 1500)
- Fig. 5. Equal separation of chromatids in 4 groups at Anaphase-II (X 1500)
- Fig. 6. 11 bivalents of A. platycarpa at Metaphase-I (X 1500)
- Fig. 7. Equal separation of 11-11 chromosomes of A. platycarpa at Anaphase-I (X 1000)
- Fig. 8. Formation of tetrads at sporad stage (X 600)
- Fig. 9. Somatic chromosomes of A. platycarpa (X 1500)
- Fig. 10. 11 bivalents of A. cajanifolia at Metaphase-I (X 1500)
- Fig. 11. 11 bivalents of A. cajanifolia at Metaphase-I (X 1500)
- Fig. 12. Equal separation of 11-11 chromosomes at Anaphase-I of A. cajanifolia (X 1500)
- Fig. 13. Somatic chromosomes of A. cajanifolia (X 1500).

PLATE - 3

10u

1 55 11 33 11 44 46 53 57 11 57 11

icans (x 1)

at diakinesis

at Metaphase

chromosomes of
(1500)

in 4 gm

2

3

4

at Metaphase

chromosomes of
(1000)

stage (x)

ycarpa (x)

at

at Metaphase

5

6

7

8

chromosomes of
(1500)

9

11

11

11

11

11

11

11

11

11

11

11

ifolia

10

11

12

pairs of chromosomes, both having 3.54μ length. Out of these two pairs, one pair of chromosome possessed sub-median primary and subterminal secondary constriction in their short arm. Length of satellite was 0.35μ . The other chromosome pair of class A was observed with median primary constriction. Class B includes 9 pairs of chromosomes having length between 2.48μ to 2.83μ , two of which have median, five submedian and two subterminal primary constriction.

Thus, total chromosome length ranged from 2.48μ to 3.54μ with total chromatin length 63.6μ and T.F. % 42.78 (Table-18).

Meiosis

Meiotic study revealed eleven bivalents at diakinesis and metaphase-I (Plate-3; Figs.10,11). Ring and rod bivalents ranged from 7-11 and 0-4 with 10.18 and 0.82 per cell respectively (Table-20). Chiasma frequency as observed at diakinesis was 21.18 per cell and 1.92 per bivalent (Table-19). At anaphase-I and II equal separation of chromosomes to the poles was observed resulting in regular tetrad formation at scored stage (Plate-3; Fig.12).

Pollen fertility was 99.7 per cent and fertile pollent size ranged from 36μ to 45μ with 43.5μ mean diameter.

Atylosia lineata (JM 3346)

Mitosis

At somatic metaphase, 22 chromosomes were observed (Plate-4; Fig.1). The chromosome complement of Atylosia lineata consists of two classes i.e. Band C. Class B includes 9 pairs of chromosomes, all having length between 2.12μ to 2.84μ . Out of these, two have median, five possessed submedian and two with sub-terminal primary constrictions. In class B,

Table - 21

Observations on somatic chromosome complements of Atylosia lineata.

| Pair No. | Class | Position of constriction | | Length of short arm in (μ) | Length of long arm in (μ) | Total chromosome length (μ) | L/S arm ratio |
|----------|-------|--------------------------|----------------|----------------------------------|---------------------------------|-----------------------------------|---------------|
| | | Pri- mary | Sec- ondary | | | | |
| 1 | B | SM | SAT | 1.06+ 0.36 | 1.42 | 2.84 | 1.66 |
| 2 | B | ST | | 0.71 | 2.13 | 2.84 | 3.0 |
| 3 | B | M | | 1.27 | 1.27 | 2.54 | 1.00 |
| 4 | B | SM | | 1.13 | 1.28 | 2.41 | 1.12 |
| 5 | B | SM | | 0.99 | 1.42 | 2.41 | 1.43 |
| 6 | B | M | SAT | 0.89+0.34 | 0.89 | 2.12 | 0.71 |
| 7 | B | SM | | 0.92 | 1.20 | 2.12 | 1.30 |
| 8 | B | ST | | 0.72 | 1.41 | 2.12 | 2.00 |
| 9 | B | SM | | 0.85 | 1.27 | 2.12 | 1.49 |
| 10 | C | M | | 0.85 | 0.85 | 1.70 | 1.00 |
| 11 | C | M | | 0.71 | 0.71 | 1.42 | 1.00 |

$$\text{T.F. \%} = \frac{21.22}{49.26} \times 100 = 43.07$$

Karyotypic formula:

$$2 \text{ B (M)} + 5 \text{ B (SM)} + 2 \text{ B (ST)} + 2 \text{ C (M)}$$

Table - 22

Chiasma frequency in Atylosia lineata

| Stage | No. of cells studied | Bivalents with | | Total Xmeta | Xmeta per cell | Xmeta per bivalent |
|------------|----------------------|----------------|-------|-------------|----------------|--------------------|
| | | 2 Xmeta | 1 Xma | | | |
| Diakinesis | 50 | 513 | 37 | 1063 | 21.26 | 1.93 |

Table - 23

Chromosome association at metaphase - 1 in Atylosia lineata

| No. of cells studied | Chromosome association at M-1 | | No. of cells per each type | Frequency per cent | Pollen fertility % |
|----------------------|-------------------------------|--------|----------------------------|--------------------|--------------------|
| | Ring II | Rod II | | | |
| 40 | 11 | 0 | 19 | 47.5 | 99.4 |
| | 10 | 1 | 6 | 15.0 | |
| | 9 | 2 | 15 | 37.5 | |
| Range | 9 - 11 | 0 - 2 | | | |
| Mean | 10.1 | 0.9 | | | |

among submedian chromosomes there was a long pair of chromosomes having secondary constriction at subterminal position with a satellite of 0.36μ in length. One pair of median chromosome of class B also possesses secondary constriction with a satellite of 2.34μ in length. Class C includes two pairs of chromosomes having length 1.42μ and 1.70μ with median primary constrictions.

Thus, the total chromosome length varied from 1.42 to 2.83μ . Length of total chromosome complement of A. lineata was 49.26μ with I.E. ± 43.07 (Table-21).

Meiosis

Meiosis showed eleven bivalents at diakinesis and metaphase-I (Plate-4; Figs.2,3). At diakinesis two pairs of chromosomes attached to the nucleolus were seen. At metaphase-I, ring and rod bivalents ranged from 0-2 with 0.9 per cell (Table-23). Chiasma frequency was 21.26 per cell and 1.93 per bivalent (Table-22). At anaphase-I and II, equal separation of chromosomes was registered. At anaphase stage regular tetrad formation was noticed resulting in higher pollen fertility (99.4%) and fertile pollen size ranged from 33μ to 42μ with 41.4μ mean diameter.

A. lineata (Jn 2039)

Mitosis

Mitotic study revealed 22 chromosomes at metaphase (Plate-4; Fig.6). The chromosome complement of Stylosia lineata consists of two classes B and C. Class B includes 6 pairs having length between 2.13μ to 2.23μ , one of which possesses median, three, submedian and two subterminal primary constrictions. Among these submedian chromosome pairs of class B, one possesses secondary constriction in its short arm with a satellite of 0.35μ in length. Class C comprised five pairs of chromosomes having length between 1.05 to 1.84μ , one of which have median and four submedian primary constrictions.

Table - 24

Observations on somatic chromosome complement of Atylosia lineata.

| Pair No. | Class | Position of constriction | | Length of short arm (u) | Length of long arm (u) | Total chromosome length (u) | L/s arm ratio |
|----------|-------|--------------------------|------------|-------------------------|------------------------|-----------------------------|---------------|
| | | Prim-ary | Sec-ondary | | | | |
| 1 | B | SM | SAT | 0.71+0.35 | 1.17 | 2.23 | 1.00 |
| 2 | B | ST | | 0.71 | 1.42 | 2.13 | 2.02 |
| 3 | B | SM | | 0.92 | 1.21 | 2.13 | 1.30 |
| 4 | B | SM | | 0.98 | 1.15 | 2.13 | 1.15 |
| 5 | B | M | | 1.06 | 1.06 | 2.13 | 1.00 |
| 6 | B | ST | | 0.71 | 1.42 | 2.13 | 2.02 |
| 7 | C | M | | 0.92 | 0.92 | 1.84 | 1.00 |
| 8 | C | SM | | 0.85 | 0.99 | 1.84 | 1.16 |
| 9 | C | SM | | 0.61 | 0.81 | 1.42 | 1.32 |
| 10 | C | SM | | 0.61 | 0.81 | 1.42 | 1.32 |
| 11 | C | SM | | 0.42 | 0.63 | 1.05 | 1.50 |

$$T.F. \% = \frac{17.7}{40.86} \times 100 = 43.31$$

Karyotypic formula:

$$1 B (M) + 3 B (SM) + 2 B (ST) + 1 C (M) + 4 C (SM)$$

Table - 25

Chiasma frequency in Atylosia lineata

| Stage | No. of cells studied | Bivalents with 2Xmata | with 1 Xma | Total xmata | Xmata per cell | Xmata per bivalent |
|------------|----------------------|-----------------------|------------|-------------|----------------|--------------------|
| Diakinesis | 50 | 520 | 30 | 1070 | 21.4 | 1.94 |

Table -26

Chromosome association at Metaphase - 1 in Atylosia lineata

| No. of cells studied | Chromosome association at M-1 | Ring II | Rod II | No. of cells per each type | Frequency per cent | Pollen fertility % |
|----------------------|-------------------------------|---------|--------|----------------------------|--------------------|--------------------|
| 75 | 11 | | 0 | 56 | 74.6 | 99.7 |
| | 10 | | 1 | 10 | 13.3 | |
| | 9 | | 2 | 3 | 3.99 | |
| | 8 | | 3 | 6 | 7.98 | |
| Range | 8-11 | | 0-3 | | | |
| Mean | 10.54 | | 0.45 | | | |

Thus total chromosome length ranged from 1.05 μ to 2.23 μ . Length of total chromosome complement of A. lineata was 40.86 and T.F. % 43.31 (Table-24).

Meiosis

Meiotic study revealed eleven bivalents at diakinesis and metaphase-I (Plate-4; Fig.4). Ring bivalents ranged from 8-11 with 10.54 per cell and rod bivalents ranged from 0-3 with 0.45 per cell (Table-26). Chiasma frequency as observed at diakinesis was 21.4 per cell and 1.94 per bivalent (Table-25). At anaphase-I and II, equal separation of chromosomes to the poles (Plate-4; Fig.5) was observed regularly. At sporad stage regular tetrad formation was observed resulting in high pollen fertility (99.7%) and fertile pollen size ranged from 36 to 42 μ with 39.0 mean diameter.

Cajanus cajan (SNT coll.)

Mitosis

At somatic metaphase 22 chromosomes (Plate-4; Fig.9) were observed. The chromosome complement of Cajanus cajan (Table-27), consists of three classes i.e. A, B and C. Class A includes only one pair of chromosomes having 3.5 μ length and a median primary constriction. Class B includes nine pairs of chromosomes having length between 2.12 to 2.83 μ , two of which have median, five submedian and two subterminal primary constriction. Class C includes one pair of chromosome having 1.4 μ length and a submedian primary constriction.

Thus, total chromosomal length ranged from 1.41 to 3.54 μ with a total chromatin length 53.16 μ and 42.0 % T.F. (Table-27).

Meiosis

Meiotic study revealed regular formation of eleven bivalents at diakinesis and metaphase-I (Plate-4; Fig.7). At

Table - 27

Observations on somatic chromosome complement of Cajanus cajan.

| Chromosome Pair No. | Class | Position of constriction | | Length of short arm in (μ) | Length of long arm in (μ) | Total chromo- some length (μ) | L/S arm ratio |
|---------------------------|-------|-----------------------------|----------------|---|--|---|---------------------|
| | | Pri- mary | Secun- dary | | | | |
| 1 | A | M | | 1.77 | 1.77 | 3.54 | 1.00 |
| 2 | B | SM | | 1.06 | 1.77 | 2.83 | 1.66 |
| 3 | B | SM | | 1.06 | 1.77 | 2.83 | 1.66 |
| 4 | B | M | | 1.41 | 1.41 | 2.82 | 1.00 |
| 5 | B | SM | | 1.06 | 1.76 | 2.82 | 1.66 |
| 6 | B | SM | | 0.92 | 1.20 | 2.12 | 1.30 |
| 7 | B | SM | | 0.92 | 1.20 | 2.12 | 1.30 |
| 8 | B | M | | 1.06 | 1.06 | 2.12 | 1.00 |
| 9 | B | ST | | 0.70 | 1.42 | 2.12 | 2.00 |
| 10 | B | ST | | 0.70 | 1.42 | 2.12 | 2.00 |
| 11 | C | SM | | 0.63 | 0.78 | 1.41 | 1.23 |

$$\text{T.F. \%} = \frac{22.58}{53.16} \times 100 = 42.04$$

Karyotypic Formula:

$$1 \text{ A (M)} + 2 \text{ B (M)} + 5 \text{ B (SM)} + 2 \text{ B (ST)} + 1 \text{ C (SM)}.$$

Table - 28

Chiasma frequency in Cajanus cajan (SNT Coll.)

| Stage | No. of cells studied | Bivalents with | | Total Xmata | Xmata per cell | Xmata per bivalent |
|------------|----------------------|----------------|-------|-------------|----------------|--------------------|
| | | 2 Xmata | 1 Xma | | | |
| Diakinesis | 50 | 508 | 42 | 1058 | 21.16 | 1.92 |

Table - 29

Chromosome association at Metaphase - 1 in Cajanus cajan (SNT Coll.)

| No. of cells studied | Chromosome association at M-1 | | No. of cells per each type | Frequency per cent | Pollen fertility % |
|----------------------|-------------------------------|--------|----------------------------|--------------------|--------------------|
| | Ring II | Rod II | | | |
| 50 | 11 | 0 | 26 | 52.0 | 99.2 |
| | 10 | 1 | 13 | 26.0 | |
| | 9 | 2 | 11 | 22.0 | |
| Range | 9 - 11 | 0 - 2 | | | |
| Mean | 10.3 | 0.7 | | | |

Metaphase-I ring bivalents ranged from 9-11 with 10.3 per cell and rod bivalents ranged from 0-2 with 0.7 per cell (Table-29). Chiasma frequency as observed at diakinesis was 21.16 per cell and 1.92 per bivalent (Table-28). At anaphase-I and II regular separation of 11-11 chromosomes to the poles (Plate-4; Fig.8) was observed. At sporad stage, regular tetrad formation was observed and high pollen fertility percentage (99.2) was recorded. Fertile pollen size ranged from 36 to 45 μ with 42.0 μ mean diameter.

Cajanus cajan (ICP 8647)

Mitosis

Mitotic metaphase of root tip cells revealed 22 chromosomes (Plate-4; Fig.14). The chromosome complement of Cajanus cajan (ICP 8647) consists of three classes i.e. A,B, and C (Table-30). Class A includes six pairs of chromosomes, one of which median, four submedian and one subterminal primary constriction. Among the submedian chromosome pairs of class A, two pairs possesses secondary constriction in short arm. Length of satellite was 0.35 μ . Class B includes four pairs of chromosomes, two of which have median, one sub-median and one subterminal primary constriction. Class C comprised only one pair of chromosome with submedian primary constriction.

Hence total chromosome length ranged from 1.77 μ to 3.24 μ with total chromatin length 67.36 μ and I.F.% 43.14.

Meiosis

Meiotic study showed formation of eleven bivalents at diakinesis and metaphase-I regularly (Plate-4; Figs. 10,11). Two bivalents were attached with nucleolus. At metaphase-I ring bivalents ranged from 8-11 with 10.03 per cell and rod bivalents ranged from 0-3 with 0.97 per cell (Table-3). Chiasma frequency was 21.06 per cell and 1.91 per bivalent (Table-31). At anaphase-I and II, regular separation of equal chromosomes to the poles (Plate-4; Fig.12) resulted in 99.3 per cent pollen fertility.

Table - 30

Observations on somatic chromosome complement of Cajanus cajan.

| Pair No. | Class | Position of constriction | | Length of short arm in (μ) | Length of long arm in (μ) | Total chromosome length (μ) | 1/S arm ratio |
|----------|-------|--------------------------|-----------|----------------------------------|---------------------------------|-----------------------------------|---------------|
| | | Primary | Secondary | | | | |
| 1 | A | SM | SAT | 1.77+0.35 | 2.13 | 4.25 | 1.00 |
| 2 | A | SM | SAT | 1.42+0.35 | 1.78 | 3.56 | 1.00 |
| 3 | A | SM | | 1.42 | 2.13 | 3.55 | 1.50 |
| 4 | A | SM | | 1.42 | 2.13 | 3.55 | 1.50 |
| 5 | A | ST | | 1.06 | 2.48 | 3.54 | 2.33 |
| 6 | A | M | | 1.77 | 1.77 | 3.54 | 1.00 |
| 7 | B | SM | | 1.06 | 1.77 | 2.83 | 1.66 |
| 8 | B | M | | 1.42 | 1.42 | 2.84 | 1.00 |
| 9 | B | ST | | 0.71 | 1.42 | 2.13 | 2.00 |
| 10 | B | M | | 1.06 | 1.06 | 2.12 | 1.00 |
| 11 | C | SM | | 0.71 | 1.06 | 1.77 | 1.49 |

$$T.P. \% = \frac{29.06}{67.36} \times 100 = 43.14$$

Karyotypic formula:

$$1 A (M) + 4 A (SM) + 1 A (ST) + 2 B (M) + 1 B (SM) + 1 B (ST) + 1 C (SM).$$

Table -31

Chiasma frequency in Cajanus cajan

| Stage | No. of cells studied | Bivalents with | | Total Xmata | Xmata per cell | Xmata per bivalent |
|------------|----------------------|----------------|-------|-------------|----------------|--------------------|
| | | 2 Xmata | 1 Xma | | | |
| Diakinesis | 50 | 302 | 28 | 632 | 21.06 | 1.91 |

Table -32

Chromosome association at Metaphase - 1 in Cajanus cajan

| No. of cells studied | Chromosome association at Metaphase - 1 | | No. of cells per each type | frequency per cent | Pollen fertility % |
|----------------------|---|--------|----------------------------|--------------------|--------------------|
| | Ring II | Red II | | | |
| 40 | 11 | 0 | 21 | 52.5 | 99.3 |
| | 10 | 1 | 8 | 20.0 | |
| | 9 | 2 | 2 | 5.0 | |
| | 8 | 3 | 9 | 22.5 | |
| Range | 8 - 11 | 0 - 3 | | | |
| Mean | 10.03 | 0.97 | | | |

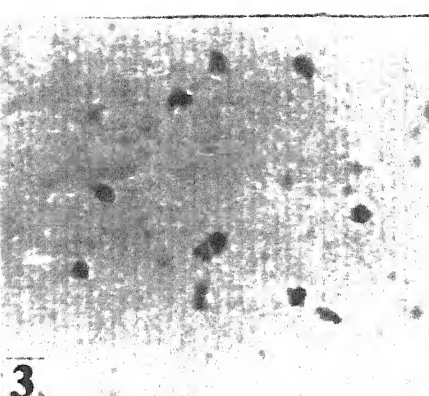
Plate - 4.

- Fig. 1. Somatic chromosomes of A. lineata (JM 3366) (X 1500)
- Fig. 2. 11 bivalents of A. lineata (JM 3366) at diakinesis (X 1500)
- Fig. 3. 11 bivalents of A. lineata (JM 3366) at Metaphase - I (X 1500)
- Fig. 4. 11 bivalents of A. lineata (JM 2639) at Metaphase - I. (X 1500)
- Fig. 5. Equal separation of 11-11 chromosomes of A. lineata (JM 2639) at Anaphase-I (X 1500)
- Fig. 6. Somatic chromosomes of A. lineata (JM 2639) (X 1500)
- Fig. 7. 11 bivalents of C. cajan (SNT Coll.) at Metaphase-I (X 1500)
- Fig. 8. Equal separation of 11-11 chromosomes of C. cajan (SNT Coll.) at Anaphase-I (X 1500)
- Fig. 9. Somatic chromosomes of C. cajan (SNT. Coll) (X 1500)
- Fig. 10. 11 bivalents of C. cajan (ICP 8647) at diakinesis (X 1500)
- Fig. 11. Eleven bivalents of C. cajan (ICP 8647) at Metaphase - I (X 1500)
- Fig. 12. Equal separation of 11-11 Chromosomes of C. cajan (ICP 8647) (X 1500)
- Fig. 13. Tetrads at sporad stage of C. cajan (ICP 8647) (X 400)
- Fig. 14. Somatic chromosomes of C. cajan (ICP 8647) (X 1500)

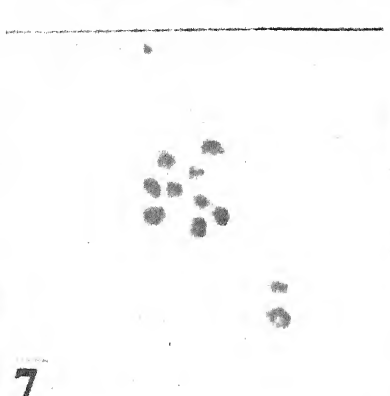
PLATE - 4

1000

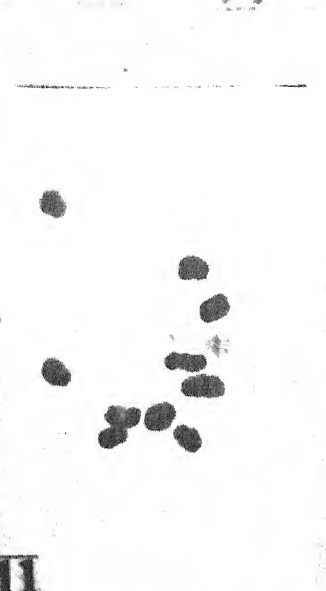
1 2 3 4 5 6 7 8 9 10 11



12 13 14 15 16 17 18 19 20 21



22 23 24 25 26 27 28 29 30 31



32 33 34 35 36 37 38 39 40 41

14

Crossability studies

Fourty one cross combinations in interspecific and intergeneric hybridization involving different Atylosia species and Cajanus cajan were attempted. Out of these, 29 cross combinations were between Atylosia spp., and 12 between Atylosia spp. and Cajanus cajan (Tables, 33,34). Crosses were attempted in both the directions. The percentage success of each cross was recorded.

Interspecific crosses:

Five cross combinations were made using Atylosia platycarpa as a pistillate parent (Table-33). In A. platycarpa x Atylosia lineata (JM 3366) cross, 50 flowers were pollinated and three pods were formed, out of three, two were seedless and one pod having two partially filled seeds which did not germinate. In the A. platycarpa x A. cajanifolia cross, 75 flowers were pollinated but no pod was formed. In A. platycarpa x A. albicans 45 crosses were attempted and no pod was formed. In both the crosses flowers shed after 2-4 days of pollination. In A. platycarpa x A. scarabaeoides cross, two mature pods were obtained and contained wrinkled seeds, which could not germinate. In A. platycarpa x A. mollis cross, 50 flowers were pollinated and three pods were obtained, two of these were seedless and one having two seeds, out of two, only one germinated and the F_1 hybrid plant was raised.

Using, A. lineata (JM 2639) as female parent, five cross combinations were attempted. In the A. lineata x A. albicans cross, 1500 flowers were pollinated and four pods were obtained, out of which two were seed less and two pods having single seed in each were obtained. Out of these two seeds one germinated and the F_1 hybrid plant was raised. In the A. lineata x A. scarabaeoides cross, 300 flowers were pollinated and no crossed pod could be obtained. In A. lineata

Table - 33
 INTERSPECIFIC hybridization in Atylosia species. (Per cent
 values in parentheses).

| Pistillate parent | Pollen parent | Total flowers pollina- ted (No.) | Pod formed (No.) | Seed- less pod (No.) | Seeded pod (No.) | Seeds obta- ined (No.) | Seeds germi- nated (No.) | F ₁ hybrid plant (No.) |
|-----------------------------------|--|---|------------------------|-------------------------------|------------------------|---------------------------------|-----------------------------------|--|
| | | | | | | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| <u>A. platycarpa</u> (JM 2873) | <u>A. lineata</u> (JM 2639) | 50 | 3 (6.0) | 2 (4.0) | 1 (2.0) | 2 (4.0) | 0 | - |
| <u>A. platycarpa</u> (JM 2873) | <u>A. calanifolia</u> (JM 2739) | 75 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>A. platycarpa</u> (JM 2873) | <u>A. mollis</u> (JM 2943) | 50 | 3 (2.0) | 2 (1.3) | 1 (0.66) | 2 (1.3) | 1 (0.66) | 1 (0.66) |
| <u>A. platycarpa</u> (JM 2873) | <u>A. scarabaeoides</u> (RJW Coll.) | 50 | 2 (4.0) | 2 (4.0) | 0 | 0 | 0 | 0 |
| <u>A. platycarpa</u> (JM 2873) | <u>A. albicans</u> (JM 2337) | 45 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>A. lineata</u> (JM 2639) | <u>A. albicans</u> (JM 2337) | 1500 | 4 (0.26) | 2 (0.13) | 2 (0.13) | 2 (0.13) | 1 (0.06) | 1 (0.06) |
| <u>A. lineata</u> (JM 2639) | <u>A. scarabaeoides</u> (RJW Coll.) | 300 | 1 (0.33) | 0 | 1 (0.33) | 1 (0.33) | 1 (0.33) | 0 |
| <u>A. lineata</u> (JM 2639) | <u>A. calanifolia</u> (JM 2739) | 50 | 1 (2.0) | 0 | 1 (2.0) | 1 (2.0) | 0 | 0 |
| <u>A. lineata</u> (JM 2639) | <u>A. volubilis</u> (JM 1984) | 1200 | 2 (0.166) | 2 (0.166) | 0 | 0 | 0 | 0 |

Contd....2.

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|----------------------------------|--|------|-------------|-------------|-------------|-------------|-------------|-------------|
| <u>A. lineata</u> (JM 2639) | <u>A. platycarpa</u> (JM 2873) | 200 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>A. albicans</u> (JM 2337) | <u>A. californica</u> (JM 2739) | 1300 | 9 (0.69) | 6 (0.46) | 3 (0.23) | 3 (0.23) | 3 (0.23) | 1 (0.07) |
| <u>A. albicans</u> (JM 2337) | <u>A. lineata</u> (JM 2639) | 500 | 1 (0.2) | 0 | 1 (0.2) | 1 (0.2) | 0 | 0 |
| <u>A. albicans</u> (JM 2337) | <u>A. platycarpa</u> (JM 2873) | 250 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>A. albicans</u> (JM 2337) | <u>A. volubilis</u> (JM 1984) | 2000 | 5 (0.25) | 0 | 0 | 0 | 0 | 0 |
| <u>A. albicans</u> (JM 2337) | <u>A. scarabaeoides</u> (RJW Coll.) | 150 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>A. volubilis</u> (JM 1984) | <u>A. albicans</u> (JM 2337) | 1000 | 15 (1.5) | 9 (0.09) | 6 (0.06) | 6 (0.06) | 5 (0.05) | 0 |
| <u>A. volubilis</u> (JM 984) | <u>A. lineata</u> (JM 2639) | 500 | 5 (1.0) | 5 (1.0) | 0 | 0 | 0 | 0 |
| <u>A. volubilis</u> (JM 1984) | <u>A. californica</u> (JM 2739) | 400 | 8 (2.0) | 8 (2.0) | 0 | 0 | 0 | 0 |
| <u>A. lineata</u> (JM 3366) | <u>A. albicans</u> (JM 2337) | 55 | 1 (1.81) | 0 (1.81) | 1 (1.8) | 1 | 0 | 0 |
| <u>A. lineata</u> (JM 3366) | <u>A. volubilis</u> (JM 1984) | 45 | 0 | 0 | 0 | 0 | 0 | 0 |

Contd.....3.

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|--|--|-----|-------------|---|-------------|-------------|-------------|-------------|
| <u>A. lineata</u> (JM 3366) | <u>A. scarabaeoides</u> (RJW coll.) | 65 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>A. lineata</u> (JM 3366) | <u>A. calanifolia</u> (JM 2739) | 85 | 2 (2.35) | 0 | 2 (2.35) | 2 (2.35) | 1 (1.17) | 1 (1.17) |
| <u>A. mollis</u> (JM 2943) | <u>A. platycarpa</u> (JM 2873) | 200 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>A. calanifolia</u> (JM 2739) | <u>A. lineata</u> (JM 2639) | 125 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>A. calanifolia</u> (JM 2739) | <u>A. albicans</u> (JM 3472) | 92 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>A. calanifolia</u> (JM 2739) | <u>A. volubilis</u> (JM 1984) | 105 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>A. calanifolia</u> (JM 2739) | <u>A. scarabaeoides</u> (RJW coll.) | 50 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>A. scarabaeoides</u> (RJW coll.) | <u>A. calanifolia</u> (JM 2739) | 50 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>A. scarabaeoides</u> (RJW coll.) | <u>A. lineata</u> (JM 2639) | 40 | 0 | 0 | 0 | 0 | 0 | 0 |

x A. cajanifolia cross, 50 flowers were pollinated and one pod was harvested having single seed which could not germinate. In A. lineata x A. volubilis (JM 1984) cross, 1200 flowers were pollinated, only two pods were obtained which were seedless. In the A. lineata x A. platycarpa cross, 200 flowers were pollinated but no pod could be harvested.

Using Atylosia albicans (JM 2337) as a pistillate parent, five cross combinations were made.

In A. albicans x A. cajanifolia cross, 1300 flowers were pollinated and 9 pods were harvested, out of which 6 were seedless and 3 pods contained single seed in each. All three seeds were germinated but two plants died in earlier stages of growth and only one F₁ hybrid plant survived having luxuriant vegetative growth. In the A. albicans x A. platycarpa cross, 250 and A. albicans x A. scarabaeoides cross, 150 flowers were pollinated and no pod was obtained in these crosses. In A. albicans x A. volubilis cross, 2000 flowers were pollinated and five pods were harvested which were seedless.

Three cross combinations were made using Atylosia volubilis (JM 1984) as a pistillate parent. In the A. volubilis x A. albicans cross, 1000 flowers were pollinated and 15 pods were harvested, out of which 9 were seedless, while 6 pods having single seed in each. Out of 6 seeds five germinated and given five plants of A. volubilis. In the A. volubilis x A. lineata (JM 2639) cross, 500 flowers were pollinated and 5 pods were harvested. These pods were seedless. In the A. volubilis x A. cajanifolia cross, 400 flowers were pollinated and 8 pods were obtained. All pods were seedless.

Four cross combinations were attempted using Atylosia lineata (JM 3366) as a pistillate parent.

In the A. lineata x A. volubilis cross 45 and A. lineata, A. scarabaeoides cross, 65 flowers were pollinated but no pod could be harvested in both of these combinations. In the A. lineata x A. albicans cross, 55 flowers were pollinated and one pod was obtained, having one seed, which was non-viable. In A. lineata x A. cajanifolia cross, 85 flowers were pollinated and two pods having single seed in each were obtained. Out of which only one could be germinated and a F_1 hybrid plant was raised.

In A. mollis x A. platycarpa cross, 200 flowers were pollinated and no pod could be harvested. All flower shed after 3-5 days of pollination. In some pollinations, pod initiation was started but these immature pods fell down after 12-16 days of pollination.

Using Atylosia cajanifolia as a female parent, four crosscombinations were made. In the A. cajanifolia x A. lineata cross, 125 flowers were pollinated and no pod could be obtained. In the A. cajanifolia x A. albicans cross, 92 flowers were pollinated but no pod could be harvested. In the A. cajanifolia x A. volubilis cross, 105 flowers were pollinated and in the A. cajanifolia x A. scarabaeoides cross, 50 flowers were pollinated but no pod could be harvested in both the crosses.

Two combinations were made using A. scarabaeoides as a female parent. In A. scarabaeoides x A. cajanifolia cross, 50 flowers were pollinated and in the A. scarabaeoides x A. lineata (JM 2639) cross, 40 flowers were pollinated but no pod could be harvested in both the crosses.

Thus, number of pollinations made in interspecific crosses ranged from 40 (A. scarabaeoides x A. lineata (JM2639)) to 2000 (A. albicans x A. volubilis) and per cent success of crossability in interspecific crosses ranged from 0.26 (A. lineata (JM 2639) x A. albicans) to 2.3 (A. lineata (JM 3366) x A. cajanifolia (R)) (Table-35).

Intergeneric crosses

Six cross combinations were made using Atylosia species as a pistillate parent and 6 cross combinations were made using Cajanus cajan (SNT Coll.) as a female parent (Table-34). This strain of Cajanus cajan was used in intergeneric hybridization because of its distinct leaf shape as oval-oblong. Observations on crossability studies in intergeneric hybridization are as follows:

In Atylosia platycarpa x Cajanus cajan cross, 1250 flowers were pollinated and 16 pods were harvested. Out of these, 12 were seedless and 4 having single seed in each but no hybrid could be obtained in this cross. In A. mollis x C. cajan cross, 80 flowers were pollinated and two pods were obtained which were seedless (Table-34). In A. volubilis x C. cajan cross, 2200 flowers were pollinated and 45 pods contained 5 seeds in total, which on germination gave plants of A. volubilis.

In A. lineata (JM 2639) x C. cajan cross, 1100 flowers were pollinated and 30 pods were obtained, out of which 25 were seedless and 5 pods having single seed in each. Out of 5 seeds, only two seeds could germinate. One F_1 plant died in earlier stages of growth and thus only one F_1 hybrid plant was obtained. In A. scarabaeoides x C. cajan cross, 500 flowers were pollinated and 3 pods were obtained (each having single seed). Out of 3 seeds, only one germinated and one F_1 hybrid plant was raised (Table-34). In A. albicans x Cajanus cajan cross, 3000 flowers were pollinated and 25 pods were obtained. Out of these, 17 pods were seedless and from remaining 8 pods, 10 seeds were obtained. Out of 10 seeds, 4 germinated and 2 plants survived given rise of two F_1 hybrids (Table-34).

Using Cajanus cajan as a pistillate parent, 6 cross combinations were made but no cross pod could be

Table - 34

INTERGENERIC CROSSES between Atylora and Cajanus cajan. (Per cent values in parentheses)

| Pistillate parent | Pollen Parent | Total flowers pollinated (No.) | Pod formed (No.) | Seedless pods (No.) | Seeds obtained (No.) | Seeds germinated (No.) | F ₁ hybrid plant (No.) |
|---------------------------------------|--------------------------------|--------------------------------|------------------|---------------------|----------------------|------------------------|-----------------------------------|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| <u>A. platycarpa</u> (JM 2873) | <u>C. cajan</u> (SNT coll.) | 1250 | 16 (1.28) | 12 (0.96) | 4 (0.32) | 4 (0.32) | 0 |
| <u>A. lineata</u> (JM 2639) | <u>C. cajan</u> (SNT coll.) | 1100 | 30 (1.57) | 25 (1.31) | 5 (0.26) | 2 (0.13) | 1 (0.05) |
| <u>A. marabaeoides</u> (RJW coll.) | <u>C. cajan</u> (SNT coll.) | 500 | 3 (0.6) | 0 | 3 (0.6) | 1 (0.2) | 1 (0.2) |
| <u>A. mollis</u> (JM 2941) | <u>C. cajan</u> (SNT coll.) | 80 | 2 (0.205) | 2 (0.20) | 0 | 0 | 0 |
| <u>A. albicans</u> (JM 2337) | <u>C. cajan</u> (SNT coll.) | 3000 | 25 (0.83) | 17 (0.56) | 8 (0.26) | 10 (10.33) | 2 (0.06) |
| <u>A. volubilis</u> (JM 1984) | <u>C. cajan</u> (SNT coll.) | 2200 | 45 (2.04) | 42 (1.92) | 3 (0.13) | 5 (0.22) | 0 |

Contd....2.

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|--------------------------------|--|-----|---|---|---|---|---|---|
| <u>C. caian</u> (SNT coll.) | <u>A. lineata</u> (JM 2639) | 200 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>C. caian</u> (SNT coll.) | <u>A. allucans</u> (JM 2337) | 350 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>C. caian</u> (SNT coll.) | <u>A. volubilis</u> (JM 1984) | 360 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>C. caian</u> (SNT coll.) | <u>A. platycarpa</u> (JM 2873) | 82 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>C. caian</u> (SNT coll.) | <u>A. mollis</u> (JM 2943) | 71 | 0 | 0 | 0 | 0 | 0 | 0 |
| <u>C. caian</u> (SNT coll.) | <u>A. scarabaeoides</u> (RJW coll.) | 92 | 0 | 0 | 0 | 0 | 0 | 0 |

harvested (Table-34).

Thus in intergeneric crosses, number of pollinations ranged from 71(C. caian x A. mollis) to 3000 (A. albicans x C. caian). The per cent success of crossability in intergeneric crosses ranged from 0.6 in the case of (A. scarabaeoides x C. caian) to 2.8 in the case of (A. lineata x C. caian)(Table-35).

STUDIES ON INTERSPECIFIC HYBRIDS:

Atylosia lineata (JM 2639) x Atylosia albicans

Morphology

Morphological observations on Atylosia lineata, Atylosia albicans and their hybrids (Table-36) are as follows:

1. Germination and first pair of leaves

Both the parents, F_1 and F_2 's showed hypogeal germinations and ovate shape of first pair of leaves.

2. Growth habit

Atylosia albicans is a twiner and Atylosia lineata is an erect shrub. The cross between these two parental plants resulted in F_1 hybrid with intermediate growth habit (Plate-10; Fig. 8). Out of 10 F_2 plants selected for the present study, one was twiner, seven erect and the rest two showed semierect growth habit.

3. Branching angle, stem and height

Primary branches of A. albicans and A. lineata formed acute angle and nearly right angle with their main stem respectively. Similar to female parent (A. lineata) F_1 hybrid exhibited nearly right angled branches along with the main stem. At 50% flowering stage, A. lineata and A. albicans possessed on an average four primary and six secondary branches; eleven primary and seventeen secondary branches respectively.

In both the parents as well as the F_1 hybrid the stem was green in colour with soft texture. During first year of their growth, A. albicans exhibited spread of 87 cm and A. lineata grew 95.0 cm. in height. The F_1 hybrid grew

upto 25 cm above the ground and afterward showed lateral spread of 91.0 cm.

Out of 10 F_2 plants, 8 exhibited acute angled primary branches and the rest 2 with nearly right angled primary branches along their main stem. The number of primary branches ranged from 3 to 9 the average being 7.51 and the number of secondary branches ranged from 7 to 15 the average being 11.23. In erect plants, the stem height ranged from 75 to 125 cm. In the twiner, spread was 79 cm. Plant height in semi-erect ones ranged from 20 to 65 cm with range of their spread from 58 to 105 cm. In general, the stem height ranged from 20 to 115 cm. with the average height of 51 cm and the plant spread ranged from 58 to 105 cm. The average being 86.5 cm.

4. Leaf

The leaflet shape in the case of A. albicans was obovate with oval leaf apices and in A. lineata lanceolate with acute leaf apices. The F_1 hybrid showed intermediate shape of leaf (Plate-5; Fig. 10) leaf surface was hairy in A. lineata, whereas, non-hairy leaf surface was the characteristic feature in A. albicans as well as F_1 hybrid. The average length and breadth of central leaflet of F_1 hybrid was 4 cm and 2.4 cm whereas, it were 5.20 and 2.0 cm in A. lineata and 4.0 cm and 3.2 cm in A. albicans. The average petiolar length in A. albicans was 4.0 cm and in A. lineata 2.4 cm while it was 4.4 cm in the F_1 hybrid.

In F_2 plants contrasting characters of leaf shape were as follows: Three plants had lanceolate, one with obovate and six were shown to have intermediate leaf shape. With regard to leaf hairiness, 9 plants had non-hairy leaf surface (Table-36). Leaf apices as oval, acute and intermediate types and leaf venation as palmately reticulate were seen in these plants.

5. Days to flowering and maturity

After sowing, bud initiation took place in 118 days and 102 days in A. albicans and A. lineata respectively. Whereas, in F_1 hybrid bud initiation started only 50 days after sowing. It was observed that time taken for 50% flowering and pod maturity took 124, 134 and 171 days; 196, 210 and 248 days in A. lineata, A. albicans and their F_1 hybrid respectively.

On an average the number of days consumed from bud initiation to flowering and from pod initiation to maturity were 13.11 and 13; 31.35 and 38 in A. lineata, A. albicans and F_1 hybrid respectively.

Duration for bud initiation ranged from 120 to 160 days in F_2 's. The days from sowing to 50% flowering ranged from 142 to 181 days. For full development of bud to flower 11 to 14 days were taken and for pod initiation to pod maturation 31 to 39 days. In F_2 's number of days for 50% pod maturity ranged from 196 to 222.

6. Flower

The colour of standard petal was yellow in A. albicans and yellow with purple straks in A. lineata. The F_1 hybrid showed yellow colour of standard petal with embeded purple streaks (Plate-5; Fig. 11). In F_1 hybrid, size of standard petal was 1.82 cm^2 as against 2.10 cm^2 in A. lineata and 2.56 cm^2 in A. albicans (Table-36). The nature of standard petal was persistent in both the parents and F_1 hybrid.

Out of 10 F_2 plants, 8 showed yellow colour of standard petal embeded with purple streaks colour. Size of the the standard petal ranged from 1.82 to 2.56 cm^2 .

7. Pod setting

Pod setting in the F_1 hybrid was 12.0 % as against 64.0% in A. lineata and 61.5% in A. albicans (Table-36). In F_2 plants pod setting percentage ranged from 10.0 to 42.5 the average being 18.20%. Some of the F_2 's met with more pod setting percentage in comparison to F_1 hybrid.

8. Pod

The colour of pod was green in both the parents as well as in F_1 . On an average the pod sizes in seed parent, pollen parent and their F_1 hybrid were 0.6, 0.96 and 0.56 cm^2 respectively. Similar to female parent, pods were hairy in the F_1 hybrid, while male parent showed non-hairy pods. Average pod thickness of F_1 hybrid was 0.38 cm as against 0.40 cm in A. lineata and 0.35 cm in A. albicans. Shattering nature of mature pods were the consistent feature in the parents as well as in the F_1 hybrid. The beak at the distal end of the pod was prominent in A. albicans and minute in A. lineata, F_1 showed intermediate character of beak on the pod.

In F_2 progeny all the plants studied were observed with green and shattering mature pods. The pod size ranged from 0.60 to 1.08 cm^2 , the average being 0.96 cm^2 . Six plants with prominent pod beak, three with minute pod beak and one with intermediate pod beak, were observed. Out of 10 F_2 plants studied, 7 comprised hairy pods and 3 non-hairy pods.

9. Ovule fertility

Percentage fertility of ovule was in the order of 33.0, 72.0 and 83.0 in F_1 hybrid, A. albicans and A. lineata. In F_2 's it ranged from 25.0 to 50.0 and the average being 51.55%.

10. Seed

Seed colour in A. lineata and the F_1 was brown with black dots, whereas, it was grey with black dots in the A. albicans. Average seed thickness in female parent, male parent and F_1 hybrid was recorded to be 0.30, 0.28 and 0.28 cm, respectively. Chambers per pod on an average were 1.82 in A. lineata, 3.0 in A. albicans and 1.30 in F_1 hybrid. The average number of seeds per pod were 1.00 in F_1 hybrid as against 1.82 in A. lineata and 2.80 in A. albicans. Similar to both the parents, F_1 hybrid possessed staphioled seeds.

In F_2 generation, 5 plants showed brown with black dotted seed coat colour and the remaining plants grey with black dotted seed coats. The seed thickness ranged from 0.23 to 0.35 cm with average seed thickness 0.30 cm.

11. Stomata

Stomatal size in A. lineata, A. albicans and the F_1 hybrids were 180, 108 and 143 μ respectively. In F_2 's stomatal size ranged from 108 to 180 μ the average being 124.2 μ .

Observations on somatic chromosome complement of Atylosia lineata x Atylosia albicans F_1 hybrid:

Somatic chromosome counts made in the root tip cells of F_1 plant revealed $2n = 22$ (Plate-5; Fig.1). Unlike the parents (A. lineata and A. albicans), most of the pairs of mitotic chromosomes were heteromorphic in the F_1 hybrid (Table-37). The class A, B and C have been contributed by Atylosia albicans and the classes A_1 , B_1 and C_1 by A. lineata. The karyotypic details are as follows.

Pair 1:

Both the chromosomes of pair 1 have submedian primary constriction and subterminal secondary constriction.

However, one chromosome differ from the other with respect to short arm, long arm and satellite length by 0.15μ , 0.1μ and 0.14μ respectively.

Pair 2:

The chromosomes of this pair appeared to be similar as they do not differ from each other in their short arm, long arm total length and position of primary constriction.

Pair 3:

This pair also comprised similar chromosomes as they do not differ with regard to position of primary constriction short arm, long arm and total length of chromosome.

Pair 4:

The chromosomes of this pair do not differ with respect to position of primary constriction but difference from each other in short arm, long arm and total length as 0.06μ , 0.05μ and 0.01μ was recorded.

Pair 5:

Again both the chromosomes of this pair appeared to be similar with regard to position of primary constriction, shortarm, long arm and total length of chromosome.

Pair 6:

Similar chromosomes formed this pair as they do not differ with regard to position of primary constriction, short arm, long arm and total chromosome length.

Pair 7:

This chromosome pair differ in short arm, long arm had total length by 0.14μ , 0.16μ and 0.02μ respectively.

These two chromosomes also differ in position of primary constriction as one chromosome possessed submedian and the other median primary constriction.

Pair 8:

Both the chromosomes differ in short arm, long arm and total length by 0.06μ , 0.22μ and 0.08μ respectively. They also differ in position of primary constriction as one chromosome was observed with submedian and the other with median primary constriction.

Pair 9:

Chromosomes of this pair do not differ in position of their primary constriction but difference was observed in their short arm, long arm and total length of 0.06μ , 0.08μ and 0.14μ respectively.

Pair 10:

Difference was observed in the short arm length and long arm length of 0.04μ and 0.04μ respectively. The total length of one chromosome resembled the other though difference in position of primary constriction exhibited as one possessed median and the other submedian primary constriction.

Pair 11:

With respect to long arm and total chromosome length, this pair of chromosomes showed difference of 0.65μ and 0.35μ respectively. Difference was also observed in position of primary constriction, while these chromosomes showed similar short arm length.

Thus total chromosome length in this hybrid ranged from 1.42μ to 3.54μ , with total length of chromosome complement 58.01μ and 41.90 T.F. %.

Meiotic studies in F_1 hybrid of *Atylosia lineata* x *Atylosia albicans*

Meiotic studies in F_1 hybrid revealed frequent formation of bivalents and univalents at diakinesis and metaphase-I (Plate-5; Fig.2). It can be seen from the table-38, that at metaphase-I ring bivalents ranged from 3-11 with 5.52 per cell and rod bivalents ranged from 0-7 with 2.48 per cell. Presence of 3 heteromorphous bivalents (Plate-5; Fig. 3) were noticed in 8.19% of PMCs. Univalents ranged from 0-16 with 4.39 univalents per cell. Maximum number of 16 univalents (Plate-5; Fig.6) were recorded in 2.34% of PMCs. The highest percentage of cells met with the chromosomal association of 8 II + 6 I (Plate-5; Fig.4). Formation of quadrivalent (Fig. 5) in 1.17 % of PMCs ranged from 0-1 with 0.14 per cell. Occurrence of loosely paired bivalents both at diakinesis as well as metaphase-I were noticed frequently. Chiasma frequency as observed at diakinesis was 11.08 per cell and 1.57 per bivalent (Table-39), which was much less in comparison to chiasma frequency observed in both the parents.

At anaphase-I, normal separation of chromosomes to the poles was recorded in 94.5% of the cells (Table-40). 3.15% of PMCs comprised 3 lagging chromosomes (Plate-5; Fig.7) and 1.05 % one lagging chromosome.

During meiotic cell division, at anaphase-II, laggards were observed in 2.5% of PMCs while in 97.5% PMCs, normal separation of chromatids to the poles was observed. At sporad stage, tetrad formation was observed in 97.11 % of cells and micronuclei (Plate 5; Fig.8) was recorded in 2.35% of PMCs (Table-41).

While female and male parent noticed with high pollen fertility, F_1 showed 38.51% fertile pollen (Plate-5;

Fig.9) grain. The size of fertile pollen ranged from 33 to 39 μ with 36.0 μ mean diameter.

Meiosis in F_2 plant progeny

Meiotic studies in 5 selected F_2 plants are as follows:

Plant No.1:

Chromosomal pairing as evidenced by bivalent formation comprised ring and rod bivalent formation at metaphase-I (Table-42). In this plant, ring bivalent ranged from 7-11 with 9.42 per cell and rod bivalents ranged from 0-4 with 1.39 per cell. A range of 0-2 univalents with 0.48 per cell was observed at metaphase-I. Chiasma frequency (Table-43) as observed at metaphase-I was 20.24 per cell and 1.87 per bivalent. At anaphase-I, one lagging chromosome was observed in 3.33% of cells while 99.66% cells showed normal separation of chromosomes to the poles (Table-44). Also at anaphase-II, normal separation of chromatids to the poles was observed in all the PMCs studied. At sporad stage, regular tetrad formation was observed. Fertile pollen size ranged from 36 to 39 μ with 37.5 μ mean diameter. Pollen fertility was 68.8% (Table-45).

Plant No.2:

At metaphase-I, other than bivalents, univalents too were frequently present (Table-42). Formation of ring bivalents ranged from 5-11 with 8.54 per cell and rod bivalents ranged from 0-4 with 1.07 per cell. Univalents ranged from 0-4 with 1.07 per cell. Univalents (Plate-5; Fig.12) ranged from 0-6 with 2.75 per cell. Maximum number of 6 univalents were observed in 17.17 PMCs. Chiasma frequency at metaphase-I was 18.17 per cell and 1.88 per bivalent (Table-43). During anaphase-I, one, two and three lagging chromosomes were observed in 1.33, 1.33 and 3.99% PMCs respectively, and the

rest 93.1% PMCs showed normal separation of chromosomes to the poles (Table-44). At anaphase-II laggards were observed in 4.29% PMCs and in 95.71 % cells equal separation of chromatids was observed (Table-45). At the sporad stage, formation of micronuclei was recorded in 3.33% cells. Fertile pollen size ranged from 36 to 42 μ with 40.5 mean diameter. Pollen fertility was 65.8%.

Plant No.3:

Chromosome associations restricted to bivalent (Plate-5; Fig.14) formation only. At metaphase-I (Table-42) ring and rod bivalents ranged from 8-11 and 0-3 with 9.85 and 1.14 per cell respectively. Chiasma frequency as observed at metaphase-I was 20.85 per cell and 1.89 per bivalent (Table-43). At anaphase-I and II, normal disjunction of chromosomes/chromatids was observed in all the PMCs studied (Table-44). At the sporad stage, regular tetrad formation was observed. Fertile pollen size ranged from 36 to 42 μ with 40.0 μ mean diameter and 78.9% pollen fertility (Table-45).

Plant No.4:

Formation of bivalents as well as univalents were noticed at metaphase-I (Table-42). Ring and rod bivalents ranged from 8-11 and 0-3 with 9.74 and 0.74 per cell respectively. Univalents (Plate-5; Fig.13) ranged from 0-2 with 0.79 per cell. Chiasma frequency as observed at metaphase-I, was 20.23 per cell and 1.92 per bivalent (Table-43). At anaphase-I ^{laggards} in 3.22% of cells and in the rest 96.77% cells normal separation of chromosomes was observed (Table-44). At anaphase-II, laggards were present in 2.0% of PMCs and in 98.0% PMCs normal separation of chromatids was registered (Table-45). At sporad stage, micronuclei in 1.42% of PMCs were recorded.

Table - 36

Morphological observations on Atylosia lineata (JM 2639), Atylosia albicans, their F_1 hybrid and F_2 segregants.

| Characters | <u>A. lineata</u> (O parent) | <u>A. albicans</u> (O parent) | F_1 (One plant) | F_2 's (10 plants) |
|-------------------------------|---------------------------------|----------------------------------|----------------------|---|
| Germination | Hypogeal | Hypogeal | Hypogeal | Hypogeal |
| Shape of first pair of leaves | Ovate | Ovate | Ovate | Ovate |
| Growth habit | Erect shrub | Twining shrub | Semierect | Erect (7) Twining (1) Semierect (2) |
| Branching | Nearly right angled | Acute angled | Nearly right angled | Acute angled (8) Right angled (2) |
| No. of primary branches | 4 | 11 | 5 | 7.51 |
| No. of secondary branches | 6 | 17 | 7 | 11.23 |
| Plant height/spread (cm) | 95.0 | 87.0 | 25/91 | 51/81.9 |
| Central leaflet: shape | Lanceolate | Obovate | Intermediate | Lanceolate (3) Intermediate (6) Obovate (1) |
| Surface | Hairy | Non-hairy | Non-hairy | Non-hairy (9) Hairy (1) |
| Length (cm) | 5.20 | 4.0 | 4.0 | 5.81 |
| Breadth (cm) | 2.00 | 3.2 | 2.4 | 2.50 |
| Venation | Palm. retic. | Palm. retic. | Palm. retic. | Palm. retic. |

| 1 | 2 | 3 | 4 | 5 |
|--|----------------------------|--------------------|----------------------------|--|
| length of petiole (cm) | 2.4 | 4.4 | 4.4 | 3.6 |
| leaf apices | Acute | Oval | Intermed- iate | Acute (3) Oval (1) Interme- diate (6) |
| Stem: | | | | |
| colour | Green | Green | Green | Green |
| woody/soft | Soft | Soft | Soft | Soft |
| Days from sowing to bud initiation | 102 | 118 | 150 | 139 |
| Days from sowing to flowering | 124 | 134 | 171 | 156 |
| Days between bud to flower | 13 | 11 | 13 | 12 |
| Days between pod initiation to pod maturation | 31 | 35 | 33 | 36 |
| Flower: | | | | |
| size of the standard petal (L x B) cm. | 1.5 x 1.4 | 1.6 x 1.5 | 1.4 x 1.3 | 1.5 x 1.4 |
| colour of the standard petal | Yellow with red stripes | Brownish yellow | Yellow with red stripes | Yellow with red stripes (8) brownish yellow (2) |
| nature of petals | Persistent | Persistent | Persistent | Persistent |
| length of style (cm) | 1.5 | 1.6 | 1.5 | 1.5 |

Contd....3.

| | 1 | 2 | 3 | 4 | 5 |
|-----------------------------|-----------------------|----------------------|----------------------|-----------------------|---------------------------|
| Pods: | | | | | |
| colour of pod | Green | Green | Green | Green | Green |
| pod (L x B) cm. | 1.5 x 0.4 | 1.6 x 0.6 | 1.6 x 0.6 | 1.4 x 0.4 | 1.6 x 0.6 |
| hairs on mature pod | Present | Absent | Absent | Present | Present (7) |
| beak of pod | Minute | Prominent | Prominent | Intermediate | Absent (3) |
| thickness of pod (cm) | 0.40 | 0.35 | 0.35 | 0.38 | Prominent (6) |
| nature of mature pods | Shattering | Shattering | Shattering | Shattering | Intermediate (1) |
| Seeds: | | | | | |
| colour of seed | Brown with black dots | Grey with black dots | Grey with black dots | Brown with black dots | Brown with black dots (5) |
| thickness of seed (cm.) | 0.30 | 0.280 | 0.280 | 0.280 | Grey with black dots (5) |
| chambers per pod | 1.94 | 3.0 | 3.0 | 1.30 | 0.300 |
| seed per pod | 1.82 | 2.89 | 2.89 | 1.00 | 1.80 |
| strophiole | Present | Present | Present | Present | 2.26 |
| Days to maturity | 186 | 210 | 210 | 248 | Present |
| pod set % | 64.0 | 61.5 | 61.5 | 12.00 | 232 |
| Ovule fertility | 83.0 | 72.0 | 72.0 | 33.33 | 18.20 |
| Stomata: | | | | | 35.00 |
| frequency (L x B) (μ) | 7.0 | 9.0 | 9.0 | 7.0 | 6.0 |
| | 15 x 12 | 12 x 9 | 12 x 9 | 12 x 9 | 13.5 x 9.2 |

(figures in parentheses are the number of F_2 plants).

Table - 37

Observations on somatic chromosome complement of Atylosia lineata (JM 2639) x Atylosia albicans F₁ hybrid.

| Ch. No. | Class | Position of constriction | | Length of short arm (μ) | Length of long arm (μ) | Total chromosome length (μ) | L/S arm ratio |
|---------|----------------|--------------------------|-----------|-------------------------|------------------------|-----------------------------|---------------|
| | | Primary | Secondary | | | | |
| 1 | A | SM | SAT | 1.27±0.49 | 1.78 | 3.54 | 1.01 |
| | A ₁ | SM | SAT | 1.42±0.35 | 1.77 | 3.54 | 1.00 |
| 2 | A | ST | | 1.06 | 2.13 | 3.19 | 2.13 |
| | A ₁ | ST | | 1.06 | 2.13 | 3.19 | 2.13 |
| 3 | B | SM | | 1.06 | 1.77 | 2.83 | 1.66 |
| | B ₁ | SM | | 1.06 | 1.77 | 2.83 | 1.60 |
| 4 | B | SM | | 1.27 | 1.56 | 2.83 | 1.22 |
| | B ₁ | SM | | 1.21 | 1.61 | 2.82 | 1.33 |
| 5 | B | SM | | 1.12 | 1.70 | 2.82 | 1.51 |
| | B ₁ | SM | | 1.12 | 1.70 | 2.82 | 1.51 |
| 6 | B | ST | | 0.71 | 2.10 | 2.81 | 2.98 |
| | B ₁ | ST | | 0.71 | 2.10 | 2.81 | 2.98 |
| 7 | B | SM | | 1.25 | 1.55 | 2.80 | 1.24 |
| | E ₁ | M | | 1.39 | 1.39 | 2.78 | 1.00 |
| 8 | B | SM | | 1.06 | 1.42 | 2.48 | 1.33 |
| | B ₁ | M | | 1.20 | 1.26 | 2.40 | 1.00 |
| 9 | B | SM | | 1.06 | 1.20 | 2.26 | 1.13 |
| | B ₁ | SM | | 1.00 | 1.12 | 2.12 | 1.12 |
| 10 | B | M | | 1.06 | 1.06 | 2.12 | 1.00 |
| | B ₁ | SM | | 1.02 | 1.10 | 2.12 | 1.00 |
| 11 | C | SM | | 0.71 | 1.06 | 1.77 | 1.49 |
| | C ₁ | M | | 0.71 | 0.71 | 1.42 | 1.00 |

$$T.F.\% = \frac{24.31}{58.01} \times 100 = 41.90$$

Karyotypic formula:

$$3A (SM) + 1A (ST) + 3B (M) + 11B (SM) + 2B (ST) + 1C (SM) + 1C (M)$$

Table - 38

Chromosome associations at Metaphase - I in Atylosia lineata x Atylosia albicans F₁ hybrid.

| No. of cells studied | Chromosomal associations at metaphase - I | | | | | No. of cells per each type | Per centage |
|----------------------|---|-----|---------|--------|------|----------------------------|-------------|
| | IV | III | Ring II | Rod II | I | | |
| 85 | 1 | - | 4 | 4 | 2 | 1 | 1.17 |
| - | - | - | 11 | - | - | 3 | 3.52 |
| - | - | - | 6 | 5 | - | 2 | 2.34 |
| - | - | - | 7 | 4 | - | 1 | 1.17 |
| - | - | - | 8 | 3 | - | 1 | 1.17 |
| - | - | - | 7 | 3 | 2 | 8 | 9.36 |
| - | - | - | 6 | 4 | 2 | 5 | 5.85 |
| - | - | - | 5 | 5 | 2 | 6 | 7.02 |
| - | - | - | 3 | 7 | 2 | 2 | 2.34 |
| - | - | - | 5 | 4 | 4 | 6 | 7.02 |
| - | - | - | 8 | 1 | 4 | 5 | 5.85 |
| - | - | - | 7 | 2 | 4 | 4 | 4.68 |
| - | - | - | 6 | 3 | 4 | 5 | 5.85 |
| - | - | - | 6 | 0 | 6 | 10 | 11.7 |
| - | - | - | 5 | 1 | 6 | 7 | 8.19 |
| - | - | - | 4 | 4 | 6 | 3 | 3.52 |
| - | - | - | 3 | 5 | 6 | 5 | 5.85 |
| - | - | - | 4 | 3 | 8 | 2 | 2.34 |
| - | - | - | 6 | - | 10 | 3 | 3.52 |
| - | - | - | 5 | - | 12 | 4 | 4.68 |
| - | - | - | 3 | - | 16 | 2 | 2.34 |
| <hr/> | | | | | | | |
| Range | 0-1 | | 3-11 | 0-7 | 0-16 | | |
| Mean | 0.014 | | 5.52 | 2.48 | 4.39 | | |

Table - 39

Chiasma frequency in Atylosia lineata, Atylosia albicans and their F_1 hybrid

| Plant | Stage | No. of cells studied | No. of quadri-valents | Bivalents with 2xmata 1xma | No. of univalents | Total xmata | xmata per cell | xmata per bivalent |
|--|------------|----------------------|-----------------------|----------------------------|-------------------|-------------|----------------|--------------------|
| <u>A. lineata</u> (♀ parent) | Diakinesis | 50 | - | 520 | 30 | 1070 | 21.4 | 1.94 |
| <u>A. albicans</u> (♂ parent) | Diakinesis | 50 | - | 518 | 32 | 1068 | 21.36 | 1.94 |
| <u>A. lineata</u> x <u>A. albicans</u> (F_1 hybrid) | Diakinesis | 50 | - | 202 | 150 | 554 | 11.08 | 1.57 |

Table - 40

Chromosome distribution at Anaphase-I in Atylosia lineata, Atylosia albicans and their F_1 hybrid.

| Plant | No. of cells studied | Normal separation | No. of laggarde | | | | Bridge |
|--|----------------------|-------------------|-----------------|-------------|-------------|---|-------------|
| | | | 1 | 2 | 3 | 4 | 5 |
| <u>A. lineata</u> | 95 | 95 (100) | - | - | - | - | - |
| <u>A. albicans</u> | 80 | 80 (100) | - | - | - | - | - |
| <u>A. lineata</u> x <u>A. albicans</u> (F_1 hybrid) | 95 | 90 (94.5) | - | 3 (1.05) | 3 (3.15) | - | 4 (1.05) |

Table - 41

Chromatid distribution at Anaphase - II in Atylosia lineata, Atylosia albicans and their F_1 hybrid

| Plant | No. of cells studied | Anaphase - II | | No. of cells studied | Quartet stage | | Pollen fertility % | fertile pollen size | |
|--|----------------------|-------------------|------------|----------------------|------------------|--------------|--------------------|---------------------|----------|
| | | Normal separation | Lag-gards | Bridge | Tetrad formation | Micro-nuclei | | Range (n) | Mean (n) |
| <u>A. lineata</u> | 80 | 80 (100) | - | - | 90 (100) | - | 99.7 | 36-39 | 37.5 |
| <u>A. albicans</u> | 100 | 100 (100) | - | - | 95 (100) | - | 98.9 | 33-39 | 36.0 |
| <u>A. lineata</u> x <u>A. albicans</u> (F_1 hybrid) | 90 | 78 (97.5) | 2 (2.5) | - | 83 (97.11) | 2 (2.35) | 58.51 | 33-39 | 36.0 |

(figure in parenthesis is per cent)

Table - 42

Chromosomal associations at Metaphase - I in Atylosia lineata x Atylosia albicans (F₂ plants)

| Plant No. | No. of cells studied | Chromosome associations at Metaphase - I | | | frequency | Per cent |
|-----------|----------------------|--|--------|------|-----------|----------|
| | | Ring II | Red II | I | | |
| 1 | 66 | 11 | - | - | 15 | 22.65 |
| | | 10 | 1 | - | 12 | 16.12 |
| | | 9 | 2 | - | 10 | 15.1 |
| | | 8 | 3 | - | 7 | 10.57 |
| | | 7 | 4 | - | 6 | 9.06 |
| | | 10 | - | 2 | 9 | 13.59 |
| | | 9 | 1 | 2 | 3 | 4.53 |
| | | 8 | 2 | 2 | 4 | 6.0 |
| Range | | 7-11 | 0-4 | 0-2 | | |
| Mean | | 9.42 | 1.39 | 0.48 | | |
| 2 | 64 | 11 | - | - | 5 | 7.8 |
| | | 10 | 1 | - | 8 | 3.12 |
| | | 9 | 2 | - | 4 | 6.25 |
| | | 8 | 3 | - | 3 | 3.72 |
| | | 6 | 4 | 2 | 4 | 6.25 |
| | | 9 | 1 | 2 | 2 | 3.12 |
| | | 10 | - | 2 | 12 | 18.72 |
| | | 8 | 2 | 2 | 3 | 3.12 |
| | | 6 | 3 | 4 | 1 | 1.56 |
| | | 9 | - | 8 | 9 | 14.04 |
| | | 8 | 1 | 4 | 5 | 7.8 |
| | | 7 | 2 | 4 | 2 | 3.12 |
| | | 8 | 0 | 6 | 4 | 6.25 |
| | | 7 | 1 | 6 | 3 | 4.68 |
| | | 6 | 2 | 6 | 2 | 3.12 |
| | | 5 | 3 | 6 | 2 | 3.12 |
| Range | | 5-11 | 0-4 | 0-6 | | |
| Mean | | 8.54 | 1.07 | 2.75 | | |

Contd....2.

- 2 -

| Plant No. | No. of cells studied | Chromosome associations at M-I | | | Frequency | Per cent |
|-----------|----------------------|--------------------------------|--------|------|-----------|----------|
| | | Ring II | Rod II | I | | |
| 3 | 41 | 11 | 0 | - | 18 | 43.90 |
| | | 10 | 1 | - | 8 | 19.44 |
| | | 9 | 2 | - | 6 | 14.63 |
| | | 8 | 3 | - | 9 | 21.87 |
| Range | | 8-11 | 0-3 | - | | |
| Mean | | 9.85 | 1.14 | | | |
| 4 | 43 | 11 | 0 | - | 15 | 34.88 |
| | | 10 | 1 | - | 5 | 11.62 |
| | | 9 | 2 | - | 6 | 13.92 |
| | | 8 | 3 | - | 5 | 11.62 |
| | | 10 | - | 2 | 5 | 11.62 |
| | | 9 | 1 | 2 | 4 | 4.64 |
| | | 8 | 2 | 2 | 3 | 6.96 |
| | | | | | | |
| Range | | 8-11 | 0-3 | 0-2 | | |
| Mean | | 9.74 | 0.74 | 0.79 | | |
| 5 | 54 | 11 | 0 | - | 21 | 38.85 |
| | | 10 | 1 | - | 15 | 27.75 |
| | | 9 | 2 | - | 10 | 18.51 |
| | | 8 | 3 | - | 3 | 5.55 |
| | | 7 | 4 | - | 5 | 9.25 |
| Range | | 7-11 | 0-4 | - | | |
| Mean | | 9.81 | 1.18 | | | |

Table - 43

Chiasma frequency in Atylosia lineata x Atylosia albicans (F₂ plants)

| Plant No. | Stage | No. of cells studied | Bivalents with | | No. of univalent | Total xmata | xmata per cell | xmata per bivalent |
|-----------|--------|----------------------|----------------|--------|------------------|-------------|----------------|--------------------|
| | | | 2xmata | 1xmata | leants | | | |
| 1 | Meta-1 | 66 | 622 | 92 | 32 | 1336 | 20.24 | 1.87 |
| 2 | Meta-1 | 64 | 547 | 69 | 176 | 1163 | 18.17 | 1.98 |
| 3 | Meta-1 | 41 | 404 | 47 | - | 855 | 20.85 | 1.89 |
| 4 | Meta-1 | 43 | 419 | 32 | 34 | 870 | 20.23 | 1.92 |
| 5 | Meta-1 | 54 | 530 | 64 | - | 1124 | 20.81 | 1.89 |

Table - 44

Chromosome distribution at Anaphase - I in Atylosia lineata x Atylosia albicans
(F₂ plants)

| plant No. | No. of cells studied | Normal separation | No. of lagging chromosomes | | | | Bridge |
|--------------|----------------------------|----------------------|----------------------------|-------------|-------------|---|--------|
| | | | 1 | 2 | 3 | 4 | |
| 1 | 60 | 58 (96.66) | 2 (3.33) | - | - | - | - |
| 2 | 75 | 70 (93.1) | 1 (1.33) | 1 (1.33) | 3 (3.99) | - | - |
| 3 | 50 | 50 (100) | - | - | - | - | - |
| 4 | 62 | 60 (96.77) | - | 2 (3.22) | - | - | - |
| 5 | 70 | 70 (100) | - | - | - | - | - |

(figures in parentheses are per cent)

Table - 45

Chromatid distribution at Anaphase - II in Atylosia lineata x Atylosia albicans
(F₂ plants)

| Plant No. | No. of cells studied | Normal separation | Laggs. | Bridge | Quartet Stage | | Pollen fertility | Fertile pollen size | |
|-----------|----------------------|-------------------|-------------|--------|----------------------|---------------|------------------|---------------------|----------|
| | | | | | No. of cells studied | Micro-nuclei. | | Range (μ) | Mean (μ) |
| 1 | 50 | 50 (100) | - | - | 80 | - | 68.8 | 36 - 39 | 37.5 |
| 2 | 70 | 67 (95.71) | 3 (4.29) | - | 60 | 2 (3.33) | 65.8 | 36 - 42 | 40.5 |
| 3 | 48 | 48 (100) | - | - | 80 | - | 78.9 | 36 - 42 | 40.0 |
| 4 | 50 | 49 (98.0) | 1 (2.0) | - | 70 | 1 (1.42) | 71.2 | 36 - 39 | 36.6 |
| 5 | 60 | 60 (100) | - | - | 75 | - | 85.6 | 36 - 42 | 38.5 |

(figures in parentheses are per cent)

PLATE - 5 (A. lineata x A. albicans)

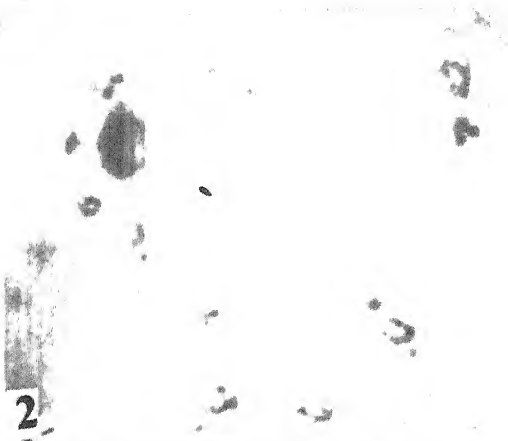
- Fig. 1. Somatic chromosome complement of A. lineata x A. albicans. F_1 hybrid (X 1500)
- Fig. 2. 9 II'_s + 4 I'_s of F_1 hybrid at diakinesis (X 1500)
- Fig. 3. 11 bivalents of F_1 hybrid at Metaphase - I showing 3 heteromörfic bivalents (4) (X 1500)
- Fig. 4. 8 II'_s = 6 I'_s at Metaphase-I of F_1 hybrid (X 1500)
- Fig. 5. 1 IV + 8 II'_s at Metaphase-I of F_1 hybrid (X 1500)
- Fig. 6. 3 II'_s + 16 I'_s at Metaphase-I of F_1 hybrid (X 1500)
- Fig. 7. Laggards at anaphase-I of F_1 hybrid (X 1500)
- Fig. 8. Micronuclei at sporad stage of F_1 hybrid (X 600)
- Fig. 9. Pollen grains of F_1 hybrid (X 600)
- Fig. 10. Leaves of female parent (A. lineata), F_1 hybrid and male parent (A. albicans) (from left to right).
- Fig. 11. Flower of A. lineata, F_1 hybrid and A. albicans (from left to right).
- Fig. 12. 9 II'_s + 4 I'_s at Metaphase-I of F_2 hybrid, plant No. 2 (X 1500)
- Fig. 13. 10 II'_s + 2 I'_s at Metaphase-I of F_2 hybrid plant, No. 4 (X 1500)
- Fig. 14. 11 II'_s at Metaphase-I of F_2 hybrid, plant No. 3 (X 1500)
- Fig. 15. Chromosomes at Anaphase-I of F_2 plant No. 4, showing one univalent away from the group.
- Fig. 16. Pollen grains of F_2 plant No. 5 showing improved fertility (X 1500)
- Fig. 17. 11 II'_s at Metaphase-I of F_2 plant No. 5. (X 1500).

PLATE - 5

104

01 11 21 31 41 51 61 71 81 91

1



3



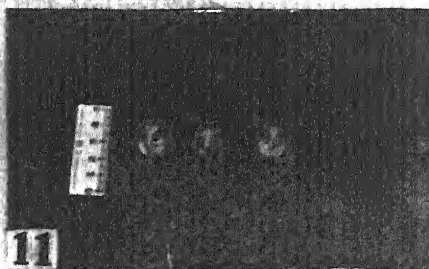
4



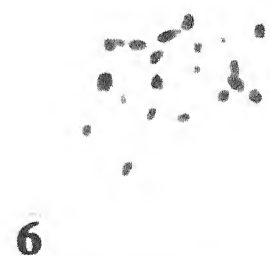
2



5



6



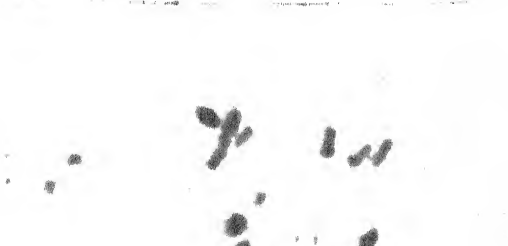
7



11



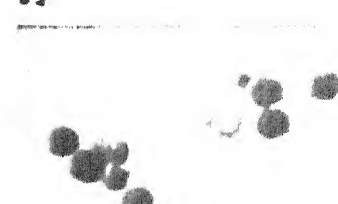
10



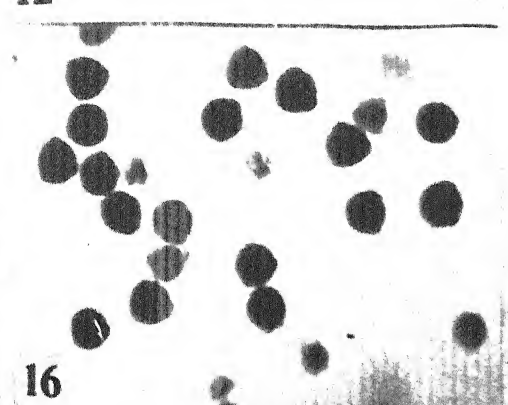
13



17



12



15



9



16



14



8



Size of fertile pollen grains ranged from 36 to 39 μ with 36.6 μ mean diameter and 71.2 percent pollent fertility.

Plant No.5:

Meiotic observations revealed ring and rod bivalents at metaphase-I, Ring bivalents ranged from 7-11 and rod bivalents 0-1 the average being 9.81 and 1.18 respectively. Bivalents (Plate-5; Fig.17) were the only association in this plant (Table-42). Chiasma frequency was 20.81 per cell and 1.89 per bivalent (Table-43). At anaphase-I and II normal disjunction of chromosomes/chromatids was recorded in all the PMCs studied (Table-44). At sporad stage, regular tetrad formation was observed (Table-45). Fertile pollen size ranged from 36-42 μ with 38.5 μ mean diameter. Pollen fertility was 85.7%.

Atylosia albicans x A. cajanifolia

Morphology

Morphological studies on Atylosia albicans, Atylosia cajanifolia, their F_1 hybrid and F_2 segregants (Table-46) are as follows.

1. Germination and first pair of leaves

Both the parents and F_1 hybrid showed hypogaeal germination. The shape of first pair of leaves was ovate in Atylosia albicans and that of Atylosia cajanifolia was lanceolate. The F_1 hybrid exhibited lanceolate shape of first pair of leaves. This indicated dominance of lanceolate shape of 1st pair of leaves over the ovate shape.

In F_2 generation, all the 20 plants studied showed hypogaeal germination. Out of 20 plants studied, 15 showed lanceolate shape of first pair of leaves and the rest 5 had ovate shape of first pair of leaves.

2. Growth habit:

Atylosia albicans is a twiner and Atylosia cajanifolia is an erect shrub. The cross between twining and erect plant types resulted in F_1 with intermediate growth habit (Plate-10; Fig.1). The F_1 hybrid showed erectness from the base and spreading in its upper part.

In F_2 generation, six plants with erect, ten plants x semierect and four plants having twining growth habit were obtained (Plate-10; Fig.2, 3).

3. Branching angle, stem and height:

Primary branches of A. albicans and A. cajanifolia formed acute angles with the main stem. Similarly, F_1 hybrid also showed acute angled primary branches. The F_1 hybrid plant exhibited luxuriant vegetative growth and beared more number of primary and secondary branches in comparison to both of the parents involved in crossing. At 50% flowering stage, A. albicans and A. cajanifolia possessed on an average eleven primary and seventeen secondary branches and four primary and seven secondary b branches respectively. The F_1 hybrid possessed 25 primary and 75 secondary branches.

The stem was noticed to be green in colour with soft texture in both the parents as well as in the F_1 hybrid.

Atylosia albicans being a twiner exhibited spread of 87.0 cm in its first year of growth. Atylosia cajanifolia is an erect shrub and showed 130 cm height in its first year of growth. The F_1 hybrid grew above the ground upto 56.0 cm and later showed spread upto 180 cm distance.

All the segregants of F_2 generation exhibited acute angled primary branches. The number of primary branches ranged from 2 to 27, the average being 17.8 and the number of secondary branches ranged from 7 to 48, the average being 28.0. In erect plants, stem height ranged from 93 cm to 131 cm. In twining plants the spread ranged from 85 cm to 112 cm. In semierect plants height ranged from 10 to 65 cm and spread ranged from 89 to 130 cm. Thus, the stem height ranged from 10 to 138 cm with the average height of 55.12 cm and 110 cm recorded in F_2 plants studied.

4. Leaf:

The central leaflet shape in the case of A. albicans was obovate with oval apices and in A. cajanifolia, lanceolate shape of central leaflets (Plate-6; Fig.1). Leaf surface of A. albicans was non-hairy, while that of A. cajanifolia was hairy, the F_1 hybrid possessed non-hairy leaf surface indicating hairiness character of leaf as recessive. The F_1 hybrid came up with vigour for length and breadth of leaves over both the parents as the average length of F_1 was 7.6 cm and average leaf breadth was 4.5 cm. Whereas in the case of A. albicans 4.2 cm average leaf length and 3.2 cm average leaf breadth was recorded and in A. cajanifolia, average leaf length was 4.9 cm and average leaf breadth was 2.2 cm. The F_1 hybrid was seen to be nearer to female parent (A. albicans) in regard to length of petiole as average petiolar length was 4.0 cm in A. albicans, 1.6 cm. in A. cajanifolia and 3.8 cm in the F_1 hybrid.

In F_2 generation contrasting characters of leaf shape segregated and out of 20 plants studied, 4 had obovate, 6 with lanceolate and 10 were shown to have intermediate leaf shape. In addition to trifoliate leaves, unifoliate, bifoliate and quadrifoliate leaves were also observed (Plate-6; Fig.12). Majority of the F_2 plants studied, showed non-hairy leaf surface (Table-46). Oval, acute and intermediate leaf apices were observed frequently. Petiolar length ranged from 1.5 cm to 4.2 cm, the average being 2.8 cm. Leaves of all the plants of

F_2 generation showed palmately reticulate venation.

5. Days to flowering and maturity:

Bud initiation, after sowing took place in 118 days and 110 days in A. albicans and A. cajanifolia respectively. While in F_1 hybrid bud initiation had started only after 124 days of sowing. The duration for 50% flowering after sowing was 136, 123 and 154 days in A. albicans, A. cajanifolia and the F_1 hybrid respectively.

On an average, the number of days consumed by bud for full development into flower and from pod initiation to pod maturity were 11, 11 and 13 and 35, 38 and 42 in A. albicans, A. cajanifolia and their F_1 hybrid respectively. After sowing 50% pod maturity was attained in 230 days, 198 days and 238 days in A. albicans, A. cajanifolia and their F_1 hybrid respectively. F_2 plants took 120 to 148 days for bud initiation after sowing and in these the days from sowing to first flush of flowers ranged from 138 to 170. Amongst 20 F_2 plants, duration for full development of flower into bud ranged from 10 to 14 days and for pod initiation to maturity it ranged from 33 to 40 days. Further it was observed that after sowing, the duration for 50% pod maturity ranged from 196 to 245 days.

6. Flower:

The colour of the standard petal was yellow in case of A. albicans and red in A. cajanifolia. F_1 hybrid had red standard petal (Plate-6; Fig.2). In F_1 hybrid, standard petal size was 2.89 cm^2 as against 2.56 cm^2 in A. albicans and 2.40 cm^2 in A. cajanifolia (Table-46). The nature of the standard petal was persistent in both the parents and the F_1 hybrid. In F_2 plants flower colour segregated in the ratio of 3:1 (15 red : 5 yellow). Standard petal size ranged from 2.25 to 2.89 cm^2 , the average being 2.56 cm^2 .

7. Pod setting:

Pod setting in F_1 hybrid was 10.0% as against 61.6% in A. albicans and 38.0% in A. cajanifolia. In F_2 segregants pod setting percentage ranged from 7.5 to 32.0%, the average being 15.2. Pod set percent was more in F_2 in comparison to F_1 hybrid (Table-46).

8. Pod:

Colour of pod in A. albicans was green and in A. cajanifolia it was brown. The F_1 hybrid resembled A. cajanifolia having brown pod colour. On an average the pod sizes in seed parent, pollen parent and their F_1 hybrid were 1.52, 2.59 and 1.57 cm^2 respectively. Similar shape of mature pods were noticed in seed parent and F_1 hybrid (Plate-6) Fig.3). Pods of A. cajanifolia were hairy with average 0.3 cm long hairs and that of A. albicans were non-hairy. The F_1 possessed hairy pods. Also the hairs of pod was reduced in length (0.12 cm). Average pod thickness of F_1 hybrid was 0.40 cm as against 0.35 cm in A. albicans and 0.50 cm in A. cajanifolia. Both the parents and F_1 hybrid showed shattering nature of mature pods with prominent beak on the distal end of the pod.

In F_2 plant progenies, segregation of pod colour was observed. Among 20 F_2 plants studied, 8 having green pod, 9 with brown pods and 3 plants having green pods with brown shades were obtained. The pod size ranged from 1.05 to 2.8 cm^2 , the average being 1.52 cm^2 . In all the F_2 plants, prominent beak of the pod and shattering nature of mature pods were noticed. Three plants with hairy pods and 17 with non-hairy pods were recorded.

9. Ovule fertility:

Percentage fertility of ovule in the order of 53.2, 72.0 and 91.0 was recorded in F_1 , A. albicans and A. cajanifolia.

In F_2 's percentage of ovule fertility range from 35.0 to 78.5 with the average of 57.0% ovule fertility.

10. Seeds:

The colour of seed in female parent was grey with black dots and in pollen parent it was red. The F_1 hybrid showed red seed colour with almost missing dots. Average seed thickness in A. albicans was 0.28 cm and in A. cajanifolia 0.40 cm while it was 0.30 cm in F_1 hybrid. Chambers per pod on an average was found to be 2.70 in A. albicans, 2.55 in A. cajanifolia and 2.10 in F_1 hybrid. The average number of seeds per pod was 0.70 in the F_1 hybrid as against 2.1 in A. albicans and 2.5 in A. cajanifolia. Both the parents and F_1 hybrid possessed seeds with prominent strophiole.

In F_2 generation variety of seed coat colours were observed viz., grey with brown dots, grey with black dots, dark red and brownish red. The seed thickness ranged from 0.23 to 4.3 cm. The 100 seed weight ranged from 3.00 to 7.00 g. All the F_2 plants studied exhibited strophioled seeds.

11. Stomata:

No marked difference in the stomatal frequency between the F_1 and the parents was noticed. However, it varied in size as 108 μ , 188 μ and 137.7 μ in seed parent, pollen parent and F_1 hybrid respectively.

Observation on somatic chromosome complement of (A. albicans x A. cajanifolia) F_1 hybrid.

Somatic chromosome counts made in the root tip cells of F_1 plant revealed $2n = 22$ (Plate-6; Fig.4). Unlike the parents (A. albicans and A. cajanifolia) most of the pairs of chromosome were heteromorphic in the F_1 hybrid (Table-47). Classes A_1 , B_1 and C_1 are contributed by

A. albicans and Classes A, B and C by A. caianifolia.

Pair 1:

It has submedian primary constriction and subterminal secondary constriction. Two chromosomes of first pair differ in total length by 0.7μ . These two chromosomes also differ in the length of short arm by 0.07μ .

Pair 2:

This pair of chromosome differ in total length, long arm length and short arm length by 0.01μ , 0.21μ and 0.22μ respectively. They also differ in position of primary constrictions as one chromosome of 2nd pair has median and the other has submedian primary constriction.

Pair 3:

Both the chromosomes of this pair has similar primary constriction and differ in long arm by 0.02μ and thus in total length also by 0.02μ .

Pair 4:

The chromosome differ in short and long arm length and total length by 0.32μ , 0.33μ and 0.01μ respectively. This chromosome pair also differ in position of primary constriction as one possess submedian and the other median primary constriction.

Pair 5:

Chromosomes of this pair are similar with respect to long and short arm length as well as total length and position of primary constriction.

Pair 6:

In this chromosomes have similar primary constriction

and short arm length but do differ in the length of long arm by 0.21μ and in total length by 0.21μ .

Pair 7:

Chromosomes of this pair are similar in position of primary constriction but differ in short arm length, long arm length and total length by 0.07μ , 0.09μ and 0.02μ respectively.

Pair 8:

Chromosomes of this pair are similar in respect of position of primary constriction, short arm, long arm and the total length.

Pair 9:

The pair of chromosomes differ in their short arm length by 0.06μ and in long arm length by 0.06μ . These chromosomes differ in position of primary constriction as one has median and the other with submedian primary constriction. Total chromosome length is same.

Pair 10:

Both chromosomes are similar with regard to position of primary constriction, but differ in short arm length by 0.76μ , and in long arm length by 0.18μ and in total length by 0.12μ .

Pair 11:

This pair of chromosomes differ in their short arm length, and long arm length by 0.17μ and 0.83μ but they do not differ in total length. They also differ in respect of position of primary constriction as one has submedian and the other has median primary constriction.

The total length of the chromosome complement of the F_1 hybrid was 55.16 μ . The total chromosome length varied from 1.78 μ to 3.5 μ with 42.6% T.F. %. The total length of the chromosome complement F_1 hybrid lies in between the total chromosome complement length of the parents.

Meiotic studies in F_1 hybrid (*A. albicans* x *A. caianifolia*)

Meiotic studies in F_1 hybrid revealed frequent formation of bivalents at diakinesis as well as at metaphase-I. It can be seen from the table-48 that at metaphase-I, ring bivalents ranged from 2-11 with 5.4 per cell and formation of rod bivalents ranged from 0-9 with 3.5 per cell. Presence of two heteromorphic bivalents were noticed frequently at metaphase-I (Plate-6; Fig.7). Other than bivalents, quadrivalents and univalents were also observed. Quadrivalents ranged from 0-1 with 0.01 per cell. Univalents ranged from 0-1 with 2.13 univalents per cell. At metaphase-I, maximum number of 10 univalents (Plate-6; Fig.6) were observed in 2.6% of pollen grain mother cells (Table-48). Whereas nine bivalents and four univalents (Plate-6; Fig.8) were recorded in 52% of pollen grain mother cells (Table-48). However, at diakinesis maximum number of 4 univalents was observed (Plate-6; Fig.5).

Chiasma frequency as can be seen from the table-49 was 17.8 chiasmata per cell and 1.73 chiasmata per bivalent.

At anaphase-I, normal separation of 11:11 chromosomes was observed in majority of the cells (Table-50) 8.3% of PMCs were shown to have two laggards (Plate-6; Fig.3) while 1.6% cells met with three laggards at anaphase-I (Table-50). At this stage, single chromatid bridge (Plate-6; Fig.10) was observed in 1.6% of PMCs. At anaphase-II laggaing chromosomes were observed in 3.0% of cells and formation of micronuclei at sperad stage in 6.52% cells (Table-51).

Pollen fertility (Plate-6; Fig.11) was recorded to be 64.0% in F_1 hybrid. Fertile pollen size ranged from 27. μ to 45 μ with 37.8 mean diameter.

Meiosis in F_2 plant progeny

Meiotic studies made in 10 selected plants of F_2 generation are as follows:

Plant No.1:

Ring bivalents ranged from 6-11 with 8.23 per cell at metaphase-I (Table-52) and rod bivalents ranged from 0-5 with 2.47 per cell. Univalents ranged from 0-2 with 0.53 univalents per cell. Chiasma frequency (Table-53) as observed at metaphase-I was 18.98 per cell with 1.76 chiasmat per bivalent. At anaphase-I (Table-54) two laggards were noticed in 7.5% of cells. In 91.8% PMCs normal separation of chromosomes to the poles was observed. At telophase-II laggards were noticed in 2.5 % PMCs. 97.5 per cent cells showed normal separation of chromatids. At sporad stage regular tetrad formation was noticed. Pollen fertility was recorded to be 64.0% in this plant. Fertile pollen size ranged from 27 μ to 33 μ with 30.3 μ mean diameter (Table-55).

Plant No. 2:

At meiotic metaphase-I, quadrivalents, bivalents and univalents were recorded (Table-52). Quadrivalent (Plate-6; Fig.13) ranged from 0-1 with 0.05 per cell. Ring bivalents ranged from 7-11 with 9.62 per cell and rod bivalents ranged from 0-3 with 1.21 per cell. Univalents ranged from 0-2 with 0.1 univalent per cell. Chiasma frequency as observed at metaphase-I was 20.45 per cell and 1.83 per bivalent. Other meiotic stages followed normal course of division. Pollen fertility percentage was 89.5. Fertile pollen size ranged from 30 to 36 μ with 32.4 μ mean diameter (Table-55).

Plant No. 3:

At metaphase-I ring bivalents ranged from 5-11 with 8.39 per cell and rod bivalents ranged from 0-4 with 2.03 per cell. Univalents (Plate-7; Fig.16) ranged from 0-4 with 0.44 per cell. Chiasma frequency was 18.8 per cell and 1.80 per bivalent (Table-53). At anaphase-I, one lagging chromosome was observed in 4.0% of cells. 96.0% cells revealed normal separation of chromosomes to the poles. At telophase-II normal separation of chromosomes was observed in 98.0% cells. In 2.0% cells laggards were recorded. At sporad stage micronuclei were noticed in 4.67% cells. The plant showed 42.8% pollen fertility and fertile pollen size ranged from 27 to 36 μ with 29.0 μ mean diameter (Table-55).

Plant No. 4:

At metaphase-I ring bivalents ranged from 2-11 with 7.73 per cell and rod bivalents ranged from 0-9 with 2.15 per cell. Univalents ranged from 0-4 with 0.84 per cell. Chiasma frequency was 17.6 per cell and 1.78 per bivalent (Table-53). At anaphase-I two lagging chromosomes were observed in 3.33% cells and in 96.2% cells normal separation of chromosomes was observed. At telophase-II, seldom appearance of one laggard (Plate-7; Fig.17) was noticed (Table-55). Formation of tetrads were normal. Pollen fertility was 73.7% and fertile pollen size ranged from 27 to 33 μ with 31.6 μ mean diameter.

Plant No. 5:

Ring bivalents ranged from 0-11 at metaphase-I , (Table-52) with 8.02 per cell and rod bivalents ranged from 0-11 with 2.29 per cell. Chiasma frequency (Table-53) observed was 18.3 per cell and 1.77 per bivalent. At anaphase-I (Table-54) one lagging chromosome was observed in 2.85% cells

and three lagging chromosomes (Plate-7; Fig.19) in 2.85% cells. The remaining 74.0% cells showed normal separation of chromosomes to the poles. At anaphase-II normal chromatid distribution was observed in all the cells studied. Pollen fertility observed was 80.6% and fertile pollen size ranged from 30 to 39 μ with 33.4 μ mean diameter (Table-55).

Plant No.6:

At metaphase-I ring bivalents ranged from 6-11 with 9.41 per cell and rod bivalents ranged from 0-5 with 1.36 per cell. Univalents ranged from 0-2 with 0.66 per cell. Frequency of chiasma was 20.19 per cell and 1.86 per bivalent. At anaphase-I normal disjunction of chromosomes to the poles (Plate-6; Fig.18) was observed in all the PMCs studied. At anaphase-II normal separation of chromatids was observed in 97.6% of cells and the rest 2.3% cells met with the formation of laggards. At sporad stage regular tetrad formation was observed. This plant showed 76.5% pollen fertility. Fertile pollen size ranged from 27 to 36 μ with 31.8 μ mean diameter (Table-55).

Plant No.7:

Ring bivalents ranged from 6-11 with 9.29 per cell and rod bivalents ranged from 0-5 with 1.48 per cell. Bivalents were the only association at metaphase-I (Table-52) in all the PMCs studied. Frequent formation of one heteromorphic bivalent was also observed (Plate-7; Fig.15). Chiasma frequency at Metaphase-I was 20.19 per cell and 1.87 per bivalent. At anaphase-I equal distribution of chromosomes to the poles was seen in all the PMCs studied. Also at anaphase-II, equal separation of chromatids and regular tetrads were observed in all the PMCs studied (Table-55). The plant exhibited 78.2% pollen fertility. Fertile pollen size ranged from 33 to 39 μ with 35.6 μ mean diameter.

Plant No.8:

Ring and rod bivalents ranged from 5-11 and 0-5 with an average 7.76 and 2.72 per cell respectively. Univalents ranged from 0-2 with 1.16 per cell. Chiasma frequency at M-I was 18.2 per cell and 1.74 per bivalent. At anaphase-I single chromatid bridge was observed in 1.8% of cells and the remaining cells (97.2%) showed normal separation of chromosomes. At anaphase-II normal separation of chromosomes. At anaphase-II normal separation of chromatids was observed in all the PMCs studied. Plant showed 79.2 per cent pollen fertility. Fertile pollen size ranged from 30 to 36 μ with 32.8 μ mean diameter (Table-55).

Plant No.9:

Ring bivalents ranged from 5-11 with 8.46 per cell and rod bivalents ranged from 0-3 with 1.17 per cell. Univalents ranged from 0-8 (Plate-6; Fig.14) with 2.70 per cell. Chiasma frequency observed at M-I was 18.0 per cell and 1.84 per bivalent. At anaphase-I two lagging chromosomes were noticed in 8.0% of PMCs and three laggards in 2.0% of PMCs. 40.0 per cent cells showed normal separation of chromosomes. Laggards were observed in 6.0% of cells and formation of micronuclei in 2.95 % cells.

Pollen fertility observed was 56.2%. Fertile size ranged from 27 to 33 μ with 31.2 μ mean diameter (Table-55).

Plant No.10:

Meiosis revealed only bivalent association of chromosomes in this plant (Table-52). Ring and rod bivalents ranged from 9-11 and 0-2 with an average 10.25 and 0.75 per cell respectively. Chiasma frequency (Table-53) at M-I was 21.25 per cell and 1.94 per bivalent. At anaphase-I equal

Table - 46

Morphological observations on Atylosia albicans, Atylosia cajanifolia their F_1 hybrid and F_2 segregants.

| Characters | P's | | | | |
|-------------------------------|-----------------------------|---------------------------|-------------------------------------|---------------------------------------|--|
| | A. <u>albicans</u> | A. <u>cajanifolia</u> | <u>F₁</u> (one plant) | <u>P₂'s</u> (20 plants) | |
| | 1 | 2 | 3 | 4 | 5 |
| Germination | | | | | |
| Shape of first pair of leaves | Hypogeal Ovate | Hypogeal Ovate | Hypogeal Lanceolate | Hypogeal Lanceolate | Hypogeal Ovate (5) Lanceol. (15) |
| Growth habit | Twining shrub | Twining shrub | Erect shrub | Semi-erect | Twining (4) Semi-erect (10) erect (6) |
| Branching | Acute angled 11 17 | Acute angled 4 7 | Acute angled 25 75 | Acute angled 25 75 | Acute angled 17.8 28.0 |
| No. of primary branches | | | | | |
| No. of secondary branches | | | | | |
| Stem: | | | | | |
| colour of stem | Green | Green | Green | Green | Green |
| woody/soft | Soft | Soft | Soft | Soft | Soft |
| dorsal leaflet: | | | | | |
| Shape | Obovate | Lanceolate | Intermediate | Intermediate | Obovate (4) Interme: (10) Lanceol. (6) |
| surface | Non-hairy | Hairy | Non-hairy | Non-hairy | Non-hairy (8) Hairy (2) |
| length (cm) | 4.2 | 4.9 | 7.6 | 7.6 | 7.91 |
| breadth (cm) | 3.2 | 2.2 | 4.5 | 4.5 | 4.62 |
| venation | palm.reti. | palm.reti. | palm.reti. | palm.reti. | palm.reti. |
| length of petiole (cm) | 4.0 | 1.6 | 3.8 | 3.8 | 2.8 |
| leaf apices | Oval | Acute | Intermediate | Intermediate | Intermediate (10) Acute (6) Oval (4) |

Contd....2.

| 1 | 2 | 3 | 4 | 5 |
|---------------------------------------|----------------------|------------|--------------------------|--------------------------|
| Days from sowing to bud initiation | 118 | 110 | 124 | 128 |
| Days from sowing to flowering | 136 | 123 | 154 | 148 |
| Days from bud to flowers | 11 | 11 | 13 | 12 |
| Days between flower to pod | 35 | 38 | 42 | 36 |
| Flower: | | | | |
| size of the standard petal (l x h) cm | 1.6 x 1.6 | 1.6 x 1.5 | 1.7 x 1.7 | 1.6 x 1.6 |
| colour of the st. petal | brownish yellow | red | red | br. yellow (5) |
| nature of petals | persistent | persistent | persistent | Red (15) |
| length of style (cm) | 1.6 | 1.6 | 1.6 | 1.6 |
| pod: | | | | |
| colour of pod | green | brown | brown | green (8) |
| | | | | brown (9) |
| | | | | br. with brown shade (3) |
| pod (L x H) cm. | 1.9 x 0.8 | 3.7 x 0.7 | 2.1 x 0.75 | 1.9 x 0.8 |
| hairs on mature pod | absent | present | present | absent (17) |
| beak of pod | prominent | prominent | prominent | present (3) |
| thickness of pod (cm) | 0.35 | 0.500 | 0.402 | 0.400 |
| nature of mature pod | shattering | shattering | shattering | shattering |
| seed: | | | | |
| colour of seed | grey with black dots | red | red, dots almost missing | grey with brown dots (9) |
| | | | | grey with black dots (6) |
| | | | | dark red (2) |
| | | | | brownish red (3) |
| thickness of seed (cm) | 0.28 | 0.400 | 0.300 | 0.305 |

Contd...3.

| 1 | 2 | 3 | 4 | 5 |
|-----------------------------|------------|-------------|---------------------------|---------------------------|
| chambers per pod (no.) | 2.70 | 2.81 | 2.10 | 2.3 |
| seeds per pod (no.) | 2.10 | 2.55 | 0.70 | 1.5 |
| strophiole | present | present | present | present |
| Days to maturity | 230 | 198 | 238 | 235 |
| Pod set (%) | 61.5 | 38.00 | 10.0 | 15.21 |
| Ovule fertility (%) | 72.00 | 91.00 | 53.2 | 57.0 |
| Stomata : | | | | |
| frequency | 9.00 | 9.0 | 8.0 | 8.2 |
| stomate (L x R) μ | 12.0 x 9.0 | 15.0 x 12.0 | 13.5 x 10.2 | 13.0 x 11.0 |
| Height/spread of plant (cm) | 87.0 | 130 | 56 - height 189-spread | 125-height 162-spread. |

(Figures in parentheses are the number of F_2 plants)

Table - 47

Observations on somatic chromosome complement of Atylosia albicans x Atylosia cajanifolia F₁ hybrid.

| S. No. | Class | Position of constriction | | Length of short arm (μ) | Length of long arm in (μ) | Total chromosome length (μ) | L/S arm ratio |
|--------|----------------|--------------------------|--------|-------------------------------|---------------------------------|-----------------------------------|---------------|
| | | Prim. | Secon. | | | | |
| 1 | A ₁ | SM | SAT | 1.42+0.35 | 1.77 | 3.54 | 1.00 |
| 2 | A | SM | SAT | 1.35+0.35 | 1.77 | 3.47 | 1.00 |
| 3 | B ₁ | M | | 1.42 | 1.42 | 2.84 | 1.00 |
| 4 | B | SM | | 1.20 | 1.63 | 2.83 | 1.35 |
| 5 | B ₁ | ST | | 0.71 | 2.12 | 2.83 | 3.04 |
| 6 | B | ST | | 0.71 | 2.10 | 2.81 | 3.00 |
| 7 | B ₁ | SM | | 1.06 | 1.71 | 2.77 | 1.61 |
| 8 | B | M | | 1.38 | 1.38 | 2.76 | 1.00 |
| 9 | B ₁ | ST | | 0.71 | 2.02 | 2.72 | 2.84 |
| 10 | B | ST | | 0.71 | 2.02 | 2.72 | 2.84 |
| 11 | B ₁ | SM | | 1.06 | 1.63 | 2.69 | 1.53 |
| 12 | B | SM | | 1.06 | 1.42 | 2.48 | 1.33 |
| 13 | B ₁ | SM | | 1.02 | 1.25 | 2.27 | 1.22 |
| 14 | B | SM | | 1.09 | 1.16 | 2.25 | 1.14 |
| 15 | B ₁ | SM | | 1.00 | 1.13 | 2.13 | 1.13 |
| 16 | B | SM | | 1.00 | 1.13 | 2.13 | 1.13 |
| 17 | B ₁ | M | | 1.06 | 1.06 | 2.12 | 1.00 |
| 18 | B | SM | | 1.00 | 1.12 | 2.12 | 1.12 |
| 19 | B ₁ | SM | | 1.01 | 1.11 | 2.12 | 1.09 |
| 20 | B | SM | | 0.71 | 1.27 | 2.00 | 1.49 |
| 21 | C ₁ | SM | | 0.72 | 1.06 | 1.78 | 1.49 |
| 22 | C | M | | 0.89 | 0.89 | 1.78 | 1.49 |

$$T.F. \% = \frac{23.0}{55.16} \times 100 = 42.60$$

Karyotypic formula:

$$2 A(SM) + 3 B(M) + 11B(SM) + 4B(ST) + 1C(M) + 1C(SM)$$

Table - 48

Chromosome associations at Metaphase-1 in Atylosia albicans
x Atylosia cajanifolia F₁ hybrid.

| No. of cells studied | Chromosome associations at M-1 | | | | No. of cells per each type | Percentage |
|----------------------------|-----------------------------------|------------|-----------|------|----------------------------------|------------|
| | IV | Ring II | Rod II | I | | |
| 74 | 1 | 6 | 1 | 4 | 1 | 1.3 |
| | - | 11 | 0 | - | 8 | 10.4 |
| | - | 6 | 5 | - | 3 | 3.9 |
| | - | 7 | 4 | - | 3 | 3.9 |
| | - | 2 | 9 | - | 1 | 1.3 |
| | - | 8 | 3 | - | 1 | 1.3 |
| | - | 10 | 1 | - | 4 | 5.2 |
| | - | 9 | 2 | - | 4 | 5.2 |
| | - | 6 | 4 | 2 | 3 | 3.9 |
| | - | 3 | 7 | 2 | 4 | 5.2 |
| | - | 8 | 1 | 4 | 2 | 2.6 |
| | - | 7 | 2 | 4 | 7 | 9.1 |
| | - | 6 | 3 | 4 | 9 | 11.7 |
| | - | 5 | 4 | 4 | 5 | 6.5 |
| | - | 4 | 5 | 4 | 6 | 7.8 |
| | - | 3 | 6 | 4 | 10 | 13.0 |
| | - | 2 | 7 | 4 | 1 | 1.3 |
| | - | 3 | 3 | 10 | 1 | 1.3 |
| | - | 2 | 4 | 10 | 1 | 1.3 |
| <hr/> | | | | | | |
| Range | 0-1 | 2 -11 | 0 -9 | 0-10 | | |
| Mean | 0.01 | 6.4 | 3.5 | 2.13 | | |

Table - 49

Chiasma frequency in Atylosia albicans, Atylosia cajaniifolia and their F_1 hybrid

| plant | stage | No. of cells studied | Bivalents with 2xmata | Univalents | Total xmata per cell | xmata per bivalent | | |
|---|-------|----------------------|-----------------------|------------|----------------------|--------------------|-------|------|
| <u>A. albicans</u> | Diak. | 50 | 518 | 32 | 0 | 1068 | 21.36 | 1.94 |
| <u>A. cajaniifolia</u> | Diak. | 50 | 509 | 41 | 0 | 1059 | 21.18 | 1.92 |
| <u>A. albicans</u> x <u>A. cajaniifolia</u> | Diak | 64 | 486 | 172 | 92 | 1144 | 17.8 | 1.73 |

Table - 50

Chromosome distribution at Anaphase-1 in Atylosia albicans, Atylosia cajaniifolia and their F_1 hybrid. (Figures in parentheses are per cent)

| Plant | No. of cells studied | Normal separation | 1 | 2 | 3 | 4 | Laggards | Chromatid bridge |
|---|----------------------|-------------------|---|---------|---------|---|----------|------------------|
| <u>A. albicans</u> | 80 | 80 (100) | - | - | - | - | - | - |
| <u>A. cajaniifolia</u> | 70 | 70 | - | - | - | - | - | - |
| <u>A. albicans</u> x <u>A. cajaniifolia</u> (F_1 hybrid) | 60 | 53 (88.3) | - | 5 (8.3) | 1 (1.6) | - | 1 (1.6) | - |

Table - 51

Chromatid distribution at Anaphase - II in Atylosia albicans, Atylosia cajaniifolia and their F_1 hybrid.

| Plant | No. of cells studied | Anaphase - II Normal separation | No. of cells studied | Sporad stage Tetrad Dyad | Pollen fertility % | Fertile size Range (n) Mean (n) |
|---|----------------------|------------------------------------|----------------------|-----------------------------|--------------------|------------------------------------|
| <u>A. albicans</u> (♀ parent) | 100 | 100 (100) | 90 | - (100) | 98.9 | 33 - 39 36.0 |
| <u>A. cajaniifolia</u> (♂ parent) | 150 | 150 (100) | 88 | - (100) | 99.2 | 36 - 42 41.5 |
| <u>A. albicans</u> x <u>A. cajaniifolia</u> (F_1 hybrid) | 100 | 97 (97.0) | 92 | - (93.47) | 64.0 (6.52) | 27 - 45 37.8 |

100

(figures in parentheses are per cent)

Table - 52

Chromosome associations at Metaphase - I of Atylosia albicans
x Atylosia cajanifolia (F₂ plants)

| Plant Nos. | 2 No. of cells studied | 3 Chromosome at M-I IV | 4 Ring II | 5 Rod II | 6 I | 7 frequency | 8 Per cent |
|---------------|---------------------------------|---------------------------------|-----------------|----------------|--------|----------------|---------------|
| 1 | 94 | - | 11 | 0 | - | 30 | 31.91 |
| | | - | 10 | 1 | - | 4 | 4.25 |
| | | - | 9 | 2 | - | 6 | 6.38 |
| | | - | 8 | 3 | - | 5 | 5.3 |
| | | - | 7 | 4 | - | 8 | 17.02 |
| | | - | 6 | 5 | - | 16 | 34.02 |
| | | - | 6 | 4 | 2 | 15 | 15.9 |
| | | - | 7 | 3 | 2 | 10 | 10.6 |

| | | | |
|-------|--------|-------|-------|
| Range | 6 - 11 | 0 - 5 | 0 - 2 |
| Mean | 8.25 | 2.47 | 0.53 |

| | | | | | | | |
|---|----|---|----|---|---|----|------|
| 2 | 37 | 1 | 7 | 1 | 2 | 2 | 5.40 |
| | | - | 11 | 0 | - | 15 | 40.5 |
| | | - | 10 | 1 | - | 6 | 16.2 |
| | | - | 9 | 1 | - | 5 | 13.5 |
| | | - | 8 | 3 | - | 9 | 24.3 |

| | | | | |
|-------|------|------|------|------|
| Range | 0-1 | 7-11 | 0-3 | 0-2 |
| Mean | 0.05 | 9.62 | 1.21 | 0.10 |

| | | | | | | | |
|---|----|---|----|---|---|----|-------|
| 3 | 63 | - | 11 | 0 | - | 18 | 28.57 |
| | | - | 10 | 1 | - | 6 | 9.52 |
| | | - | 9 | 2 | - | 10 | 15.8 |
| | | - | 8 | 3 | - | 12 | 18.96 |
| | | - | 7 | 4 | - | 8 | 12.64 |
| | | - | 6 | 4 | 2 | 4 | 6.32 |
| | | - | 5 | 4 | 4 | 5 | 7.9 |

| | | | |
|-------|------|------|------|
| Range | 5-11 | 0-5 | 0-4 |
| Mean | 8.39 | 2.03 | 0.44 |

Contd..

- 2 -

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|----|---|----|---|---|----|-------|
| 4 | 52 | - | 11 | - | - | 18 | 34.56 |
| | | - | 10 | 1 | - | 2 | 3.84 |
| | | - | 9 | 2 | - | 4 | 7.69 |
| | | - | 6 | 5 | - | 6 | 11.52 |
| | | - | 5 | 6 | - | 5 | 9.6 |
| | | - | 4 | 7 | - | 1 | 1.92 |
| | | - | 2 | 9 | - | 2 | 3.84 |
| | | - | 9 | 1 | 2 | 6 | 11.52 |
| | | - | 8 | 1 | 4 | 5 | 9.6 |
| | | - | 7 | 2 | 4 | 3 | 5.76 |

| | | | | |
|-------|---|------|------|------|
| Range | - | 2-11 | 0-9 | 0-4 |
| Mean | | 7.73 | 2.15 | 0.84 |

| | | | | | | | |
|---|----|---|----|----|---|----|-------|
| 5 | 44 | - | 11 | 0 | - | 17 | 38.59 |
| | | - | 9 | 2 | - | 15 | 34.05 |
| | | - | 7 | 4 | - | 5 | 11.35 |
| | | - | 5 | 6 | - | 3 | 6.81 |
| | | - | 4 | 7 | - | 2 | 4.54 |
| | | - | 3 | 8 | - | 1 | 2.27 |
| | | - | - | 11 | - | 1 | 2.27 |

| | | |
|-------|------|------|
| Range | 0-11 | 0-11 |
| Mean | 8.02 | 2.29 |

| | | | | | | | |
|---|----|---|----|---|---|----|-------|
| 6 | 36 | - | 11 | 0 | - | 15 | 41.55 |
| | | - | 9 | 2 | - | 6 | 16.66 |
| | | - | 8 | 3 | - | 3 | 8.33 |
| | | - | 9 | 1 | 2 | 8 | 22.16 |
| | | - | 6 | 5 | 2 | 4 | 11.08 |

| | | | |
|-------|------|------|------|
| Range | 6-11 | 0-5 | 0-2 |
| Mean | 9.41 | 1.36 | 0.66 |

- 3 -

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|----|---|----|---|---|----|-------|
| 7 | 37 | - | 11 | 0 | - | 18 | 48.6 |
| | | - | 10 | 1 | - | 6 | 16.20 |
| | | - | 7 | 4 | - | 8 | 21.60 |
| | | - | 6 | 5 | - | 5 | 13.51 |

| | | |
|-------|------|------|
| Range | 6-11 | 0-5 |
| Mean | 9.29 | 1.48 |

| | | | | | | | |
|---|----|---|----|---|---|----|------|
| 8 | 50 | - | 11 | - | - | 10 | 20.0 |
| | | | 10 | 1 | - | 6 | 12.0 |
| | | | 9 | 2 | - | 5 | 10.0 |
| | | | 6 | 4 | 2 | 12 | 24.0 |
| | | | 7 | 3 | 2 | 8 | 16.0 |
| | | | 5 | 5 | 2 | 9 | 18.0 |

| | | | |
|-------|------|------|------|
| Range | 5-11 | 0-5 | 0-2 |
| Mean | 7.76 | 2.72 | 1.16 |

| | | | | | | | |
|---|----|---|----|---|---|----|-------|
| 9 | 41 | - | 11 | - | - | 15 | 36.45 |
| | | - | 10 | 1 | - | 3 | 7.29 |
| | | - | 9 | 2 | - | 3 | 7.29 |
| | | - | 8 | 1 | 4 | 3 | 7.29 |
| | | - | 7 | 2 | 4 | 2 | 4.86 |
| | | - | 6 | 3 | 4 | 5 | 12.15 |
| | | - | 6 | 2 | 6 | 3 | 7.29 |
| | | - | 6 | 1 | 8 | 3 | 7.29 |
| | | - | 5 | 2 | 8 | 4 | 9.72 |

| | | | |
|-------|------|------|------|
| Range | 5-11 | 0-3 | 0-8 |
| Mean | 8.46 | 1-17 | 2.78 |

| | | | | | | | |
|----|----|---|----|---|---|----|------|
| 10 | 60 | - | 11 | - | - | 30 | 49.9 |
| | | - | 10 | 1 | - | 15 | 24.9 |
| | | - | 9 | 2 | - | 15 | 24.9 |

| | | | |
|-------|-------|------|---|
| Range | 9-11 | 0.2 | - |
| Mean | 10.25 | 0.75 | |

Table - 53

Chiasma frequency at M-1 in Atylosia albicans x Atylosia cajanifolia (F_2 plants)

| Plant Nos. | No. of cells studied | Quadri-valents 4 Xma | Bivalents with | | | Uni-vale-nts | Total Xmata | Xmata per cell | Xmata per biva-lent |
|------------|----------------------|----------------------|----------------|------|-----|--------------|-------------|----------------|---------------------|
| | | | 2Xmata | 1Xma | | | | | |
| 1 | 94 | - | 776 | 233 | 50 | 1785 | 18.98 | 1.76 | |
| 2 | 37 | 2 | 356 | 45 | 4 | 757 | 20.45 | 1.88 | |
| 3 | 63 | - | 529 | 128 | 28 | 1186 | 18.8 | 1.80 | |
| 4 | 52 | - | 402 | 112 | 44 | 916 | 17.6 | 1.78 | |
| 5 | 44 | - | 353 | 101 | - | 807 | 18.34 | 1.77 | |
| 6 | 36 | - | 339 | 49 | 24 | 727 | 20.19 | 1.87 | |
| 7 | 37 | - | 344 | 55 | - | 743 | 20.0 | 1.86 | |
| 8 | 50 | - | 388 | 136 | 58 | 912 | 18.2 | 1.74 | |
| 9 | 41 | - | 346 | 48 | 114 | 740 | 18.0 | 1.87 | |
| 10 | 60 | - | 615 | 45 | - | 1275 | 21.25 | 1.94 | |

Table - 54

Chromosome distribution at Anaphase-1 in Atylosia albicans
 x Atylosia cajanifolia (F₂ plants)

| Plant | No. of cells studied | Normal separation | Laggards | | | | Chromatid bridge | |
|-------|----------------------|-------------------|-------------|-------------|-------------|---|------------------|--------|
| | | | 1 | 2 | 3 | 4 | single | double |
| 1 | 65 | 60 (91.8) | - | 5 (7.6) | - | - | - | - |
| 2 | 40 | 40 (100) | - | - | - | - | - | - |
| 3 | 50 | 48 (96.0) | 2 (4.0) | - | - | - | - | - |
| 4 | 60 | 58 (96.2) | - | 2 (3.33) | - | - | - | - |
| 5 | 35 | 33 (94.0) | 1 (2.85) | - | 1 (2.85) | - | - | - |
| 6 | 25 | 25 (100) | - | - | - | - | - | - |
| 7 | 50 | 50 (100) | - | - | - | - | - | - |
| 8 | 55 | 54 (97.2) | - | - | - | - | 1 (1.8) | - |
| 9 | 50 | 45 (90.0) | - | 4 (8.0) | 1 (2.0) | - | - | - |
| 10 | 42 | 42 (100) | - | - | - | - | - | - |

(figures in parentheses are per cent)

Table - 55

Chromatid distribution at Anaphase-II in Atylosia albicans x Atylosia cajanifolia (F₂ plants)
(figures in parentheses are per cent)

| Plant no. | No. of cells studied | ANAPHASE-2 | | No. of cells studied | Sporad stage | | Pollen fertility % | fertile pollen size | |
|-----------|----------------------|--------------|------------|----------------------|---------------|-------------|--------------------|---------------------|----------|
| | | Normal | Lag-gards | | Tetrad | dyad | | Range (u) | Mean (u) |
| 1 | 40 | 39 (97.5) | 1 (2.5) | 95 | 95 (100) | - | 66.8 | 27 - 33 | 30.3 |
| 2 | 50 | 50 (100) | - | 92 | 92 (100) | - | 89.5 | 30 - 36 | 32.4 |
| 3 | 48 | 47 (98.0) | 1 (2.0) | 76 | 71 (93.42) | 5 | 72.8 | 27 - 36 | 29.9 |
| 4 | 55 | 54 (98.1) | 1 (1.8) | 81 | 81 (100) | - | 73.7 | 27 - 33 | 31.6 |
| 5 | 40 | 40 (100) | - | 85 | 85 (100) | - | 80.6 | 30 - 39 | 33.4 |
| 6 | 42 | 41 (97.6) | 1 (2.3) | 70 | 70 (100) | 7 | 76.5 | 27 - 36 | 31.8 |
| 7 | 30 | 30 (100) | - | 46 | 46 (100) | - | 78.2 | 33 - 39 | 35.6 |
| 8 | 35 | 35 (100) | - | 78 | 78 (100) | - | 79.2 | 30 - 36 | 32.8 |
| 9 | 50 | 47 (94.0) | 3 (6.0) | 60 | 57 (97.15) | 3 (2.85) | 56.2 | 27 - 33 | 31.2 |
| 10 | 40 | 40 (100) | - | 90 | 90 (100) | - | 91.8 | 30 - 36 | 31.5 |

PLATE - 6 (A. albicans x A. cajanifolia)

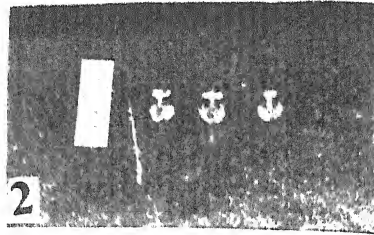
- Fig. 1. Leaves of A. albicans, F_1 hybrid, A. cajanifolia
(from left to right)
- Fig. 2. Flower of A. albicans, F_1 hybrid, A. cajanifolia
(from left to right).
- Fig. 3. Pods of A. albicans, F_1 hybrid, A. cajanifolia
(from left to right)
- Fig. 4. Somatic chromosome complement of A. albicans x
A. cajanifolia F_1 hybrid (x 1500)
- Fig. 5. 9 II'_S + 4 I'_S at diakinesis of F_1 hybrid (x 1100)
- Fig. 6. 7 II'_S 8 I'_S at Metaphase-I of F_1 hybrid (x 1500)
- Fig. 7. 11 II'_S at Metaphase-I of F_1 hybrid showing
2 heteromorphic bivalents (↑) (x 1500).
- Fig. 8. 9 II'_S + 4 I'_S at Metaphase-I of F_1 hybrid (x 1500)
- Fig. 9. 3 Leagards at Anaphase-I of F_1 hybrid (x 1500)
- Fig. 10. Bridge at Anaphase-I of F_1 hybrid (x 1500)
- Fig. 11. Pollen grains of F_1 hybrid (x 600)
- Fig. 12. Branch of F_2 plant showing bifoliate, trifoliate
and quadrifoliate leaves.
- Fig. 13. 1 IV + 8 II'_S + 2 I'_S at Metaphase-I of F_2
hybrid plant No. 2 (x 1500)
- Fig. 14. 7 II'_S + 8 I'_S at Metaphase-I of F_2 hybrid
Plant No. 5 (x 1500).

PLATE - 6

10μ

4

1



3



5

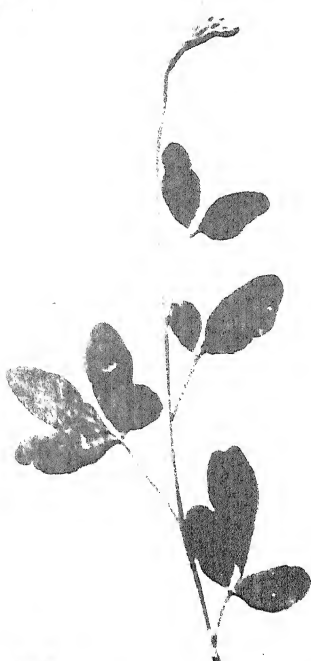
6

7

10

8

13



11

14

PLATE - 7 (15-19 A. albicans x A. cajanifolia)

- Fig. 15. 11 II'_s at Metaphase-I of F₂ hybrid plant No. 4 showing 2 heteromorphic bivalents () (x 1500)
- Fig. 16. 10 II'_s + 2 I'_s at Metaphase-I of F₂ hybrid plant No. 3 (x 1500)
- Fig. 17. Delayed separation of bivalent at anaphase of F₂ hybrid plant No. 4 (x 1500)
- Fig. 18. Equal separation of chromosomes at early Anaphase-I of F₂ hybrid plant No. 3 (x 1500)
- Fig. 19. Laggards at Anaphase-II of F₂ hybrid plant No. 5 (x 1500)

PLATE - 7 (Fig. 1-8 A. lineata x A. cajanifolia)

- Fig. 1. Leaves of A. lineata, F₁ hybrid and A. cajanifolia (From left to right)
- Fig. 2. Flower of A. lineata, F₁ hybrid and A. cajanifolia (From left to right)
- Fig. 3. Pods of A. lineata, F₁ hybrid, A. cajanifolia (From left to right)
- Fig. 4. Somatic chromosome complement of A. lineata x A. cajanifolia (x 1500)
- Fig. 5. 10 II'_s + 2 I'_s at diakinesis of F₁ hybrid (x 1500)
- Fig. 6. 11 II'_s at Metaphase-I of F₁ hybrid (x 1000)
- Fig. 7. 10 II'_s + 2 I'_s at Metaphase of F₁ hybrid showing precocious separation of 2 bivalents (x 1000)
- Fig. 8. 3 II'_s + 10 I'_s at Metaphase - I of F₁ hybrid (x 1500)

PLATE - 7

100

15

16

17

18

19

1

2

3

5

6

7

8

4

11 11 11 11 11 11 11 11 11 11 11

separation of chromosomes was observed regularly in all the PMCs studied (Table-54). Also, at anaphase-II, equal separation of chromatids to the poles was observed, indicating thereby normal meiotic cell division. This plant showed 91.8 per cent pollen fertility. Fertile pollen size ranged from 30 to 46 μ with 31.5 μ mean diameter (Table-55).

Atylosia lineata (JM 3366) x Atylosia cajanifolia

Morphology

Morphological observations on A. lineata, A. cajanifolia and their hybrids (Table-56) are as follows:

1. Germination and first pair of leaves:

Both the parents, F_1 and F_2 's showed hypogeal germination. Shape of first pair of leaves of A. lineata was ovate while that of A. cajanifolia was lanceolate. Similar to male parent (A. cajanifolia), F_1 hybrid showed lanceolate shape of first pair of leaves indicating dominance of lanceolate shape over ovate shape. Amongst 12 F_2 s studied, nine had lanceolate and 3 had ovate shape of first pair of leaves.

2. Growth habit:

Both the parents and their hybrids showed erect growth habit (Plate-10; Fig. 8,9, and 10).

3. Branching angle, stem and height:

Primary branches of A. lineata formed nearly right angle and that of A. cajanifolia, acute angles with their main stem. Similar to female parent (A. lineata) F_1 hybrid

exhibited nearly right angled branches on its main stem. At 50 per cent flowering stage, A. lineata and A. cajanifolia possessed on an average four primary and five secondary branches; five primary and thirteen secondary branches respectively. And the F_1 hybrid comprised eleven primary and seventeen secondary branches.

In both the parents as well as the F_1 hybrid the stems were green in colour with soft texture. During first year of growth, A. lineata exhibited height of 95 cm and A. cajanifolia grew above the ground upto 112 cm. The F_1 hybrid showed 185 cm height which was more in comparison to the parental plant's height.

Out of twelve F_2 plants, seven exhibited acute angled primary branches and the remaining five nearly right angled primary branches on their main stem. The number of primary branches ranged from 5 to 16, the average being 9.21 and the number of secondary branches ranged from 9 to 21, the average being 12.62. In erect plants, the stem height ranged from 97 cm to 148 cm with 121 cm average height.

Leaf:

Similar to both the parents, F_1 hybrid showed lanceolate leaf shape with acute leaf apices (Plate-7; Fig. 1). Leaf surface was hairy in both the parents as well as F_1 hybrid. The average length and breadth of central leaflet of F_1 hybrid was 8.0 cm and 3.2 cm, whereas, it were 5.5 and 2.5 cm in A. lineata and 5.2 and 2.4 cm in A. cajanifolia. The average petiolar length in A. lineata was 1.3 cm in A. cajanifolia 1.6 cm, while it was 1.9 cm in the F_1 hybrid.

All the F_2 plants showed lanceolate shape of leaves with acute leaf apices. All the F_2 's showed hairy leaf surface

and palmately reticulate venation. In these F_2 plants, length and breadth of central leaflet ranged from 4.5 to 9.2 cm and 2.3 to 3.8 cm respectively. The petiolar length ranged from 2.3 to 4.0 cm with 3.41 cm average petiolar length.

Days to flowering and maturity

After sowing bud initiation took 101 and 102 days in A. lineata and A. cajanifolia respectively, whereas, in F_1 hybrid bud initiation started only 145 days after sowing. Days to 50% flowering were observed to be 124, 122 and 165 in A. lineata, A. cajanifolia and F_1 hybrid respectively. Days to 50% pod maturity were recorded as 196, 195 and 218 in A. lineata, A. cajanifolia and F_1 hybrid respectively. On an average, the number of days taken for bud to full development into flower and pod initiation to pod maturity were 12, 11 and 12; 34, 32 and 38 in A. lineata, A. cajanifolia and F_1 hybrid respectively.

In twelve F_2 's studied, duration of bud initiation ranged from 100 to 142 and the days from sowing to flowering ranged from 125 to 160. For full development of bud into flower 10-15 days were taken and for pod initiation to pod maturation 33 to 44 days. The number of days consumed for 50% pod maturation ranged from 183 to 120 days.

Flower:

The colour of standard petal was yellow in A. lineata and red in A. cajanifolia. The F_1 hybrid showed red colour of standard petal (Plate-7; Fig.2). In F_1 hybrid size of standard petal was 2.88 cm^2 as against 2.4 cm^2 in A. lineata and 2.56 cm^2 in A. cajanifolia (Table-56). The nature of standard petal was persistent in both the parents and F_1 hybrid. In F_2 generation, Out of 12 F_2 's plants studied 3 had yellow and 9 had red colour of standard petals. Size

of the standard petal ranged from 2.40 cm^2 to 2.72 cm^2 , the average being 2.56 cm^2 . All the F_2 's observed were with persistent standard petal.

Pod setting:

Pod setting in the F_1 hybrid was 4.0% in A. cajanifolia (Table-56). In F_2 plants pod setting percentage ranged from 16.2 to 51.0, the average being 32.65%. All the F_2 's met with more pod setting percentages in comparison to F_1 hybrid.

Pod:

Colour of pod was green in A. lineata and brown in A. cajanifolia. Similar to male parent (A. cajanifolia) F_1 hybrid showed pods of brown colour indicating dominance of brown colour of pod over green pod colour. On an average the pod sizes in seed parent, pollen parent and their F_1 hybrid were 3.8, 2.32 and 3.5 cm^2 respectively. Similar to both the parents, the pods were having in F_1 hybrid. Pods of A. lineata were observed with minute beaks and that of A. cajanifolia with prominent beaks. While in F_1 hybrid intermediate type of pod beak was noticed (Plate-7; Fig.3). Pod shape characteristic of the F_1 was nearer to female parent. Average pod thickness of F_1 hybrid was 0.49 cm, as against 0.42 cm in A. lineata and 0.58 cm in A. cajanifolia. Shattering nature of mature pods was the consistent feature in the parents as well as in the F_1 hybrid.

In F_2 progeny, all the plants studied were observed with hairy pods. Amongst 12 F_2 plants, 6 had green, 2 brown and 4 green with brown shade pods. The pod size ranged from 0.9 cm^2 to 2.87 cm^2 , the average being 3.6 cm^2 . The pod thickness ranged from 0.33 cm to 0.55 cm with 0.48 cm average pod thickness. Four plants with minute pod beaks and eight with prominent pod beaks were observed. While nature of mature pods was shattering in both the parents and F_1 hybrid,

one F_2 plant met with non-shattering nature of mature pods. In remaining 11 F_2 plants, shattering nature of mature pods were observed.

Ovule fertility:

Percentage fertility of ovule was in the order of 30.0, 84.0 and 92.0 in F_1 hybrid, A. lineata and A. cajanifolia respectively. In F_2 's it ranged from 23.2 to 67.2 per cent, the average being 45.0 per cent.

Seed:

Seed colour of A. lineata was brown with black dots and in A. cajanifolia it was red. F_1 hybrid showed seed colour of brown with almost missing dots. Average seed thickness in F_1 was 0.41 cm as against 0.31 cm in A. lineata and 0.432 in A. cajanifolia. Chambers per pod, on an average was found to be 2.05, 2.90 and 1.90 in seed parent, pollen parent and F_1 hybrid respectively. The number of seeds per pod was 1.7 in A. lineata, 2.8 in A. cajanifolia and 1.3 in F_1 hybrid. Similar to both the parents, F_1 hybrid possessed strophioled seeds.

In F_2 generation six plants showed brown with black dotted seed coat colour, two complete red, one light brown and three dark brown colour of seed coats. The seed thickness ranged from 0.30 to 0.52 cm, the average being 0.426 cm. Chambers and seeds per pod ranged from 1 to 4 and 0.6 to 2.5, the average being 1.95 chambers per pod and 1.50 seeds per pod (Table-56). All the F_2 's possessed strophioled seeds.

Stomata:

Stomatal sizes in A. lineata, A. cajanifolia and their F_1 hybrid (Plate-8; Fig.12) were 108 μ , 270 μ and 180 μ respectively. In F_2 's stomatal sizes ranged from 108 μ to 270 μ the average being 183.0 μ (Table-56).

Atylosia lineata x Atylosia cajanifoliaCytologya) Mitosis:

The number of somatic chromosomes counted at metaphase was $2n = 22$ (Plate-7; Fig. 4). On the basis of total chromosome length, the somatic chromosome complement of F_1 hybrid were grouped into three classes (Table-57). The classes, A, B and C contributed by A. cajanifolia and A_1 , B_1 and C_1 by A. lineata. In the F_1 , the somatic chromosomes were linearly arranged in pairs as per their length in descending order, the karyotypic description of each chromosome pair is as follows:

Chromosome pair 1:

Both the chromosomes differed from each other in short arm and long arm length by 0.35μ and 0.35μ respectively. These two chromosomes are similar with regard to position of primary constriction, secondary constriction and total chromosome length.

Chromosome pair 2:

Both the chromosomes possessed similar position of primary constriction and short arm, long arm and total chromosome length.

Chromosome pair 3:

Both the chromosomes showed subterminal primary constriction but differed in short arm, long arm and total length by 0.02μ , 0.04μ and 0.02μ respectively.

Chromosome pair 4:

These two chromosomes differed in long arm and total length by 0.35μ and 0.35μ respectively. Difference was also observed in position of primary constriction as one chromosome

had submedian and the other median primary constriction. They possessed similar short arm length.

Chromosome pair 5:

Chromosome of this pair differed in short arm and long arm length by $0.70\ \mu$ and $0.70\ \mu$ respectively. The total chromosome length was similar in these two chromosomes. They also differed in position of primary constriction, as one chromosome possessed subterminal and the other submedian primary constriction. One chromosome of this pair possessed subterminal secondary constriction (Table-57).

Chromosome pair 6:

The two chromosomes of this pair differed slightly in their short arm, long arm and total length by $0.01\ \mu$, $0.01\ \mu$ and $0.02\ \mu$ respectively. They had similar position of primary constriction.

Chromosome pair 7:

Both the chromosomes differed in short arm, long arm and total length by $0.39\ \mu$, $0.91\ \mu$ and $0.02\text{-}\mu$ respectively. Difference was also noticed in position of primary constriction as one chromosome had submedian and the other had median primary constriction.

Chromosome pair 8:

Difference was noticed in long arm and total chromosome length by $0.36\ \mu$ and $0.36\ \mu$ respectively. Length of short arm was similar but difference in the position of primary constriction was noticed.

Chromosome pair 9:

Chromosome differed from each other in short arm and

long arm length by 0.14 μ and 0.14 μ respectively. One chromosome of this pair possessed submedian and the other subterminal position of primary constriction, however, they showed similarity in total chromosome length.

Chromosome pair 10:

Both the chromosomes of this pair differed from each other in short arm, and total length by 0.34 μ and 0.34 μ respectively. They had equal long arm length with different position of primary constriction.

Chromosome pair 11:

Both the chromosomes of this pair appeared to be homomorphic with regard to position of primary constriction, short arm, long arm and total length of chromosomes.

Thus in this hybrid, total chromosome length ranged from 1.7 μ to 3.55 μ and the cumulative length of chromosome complement was observed to be 60.49 μ with 42.60% T.F. (Table-57).

Meiotic studies in F_1 hybrid of *Atylosia lineata* x *Atylosia cajanifolia*.

Meiotic studies in F_1 hybrid revealed frequent formation of bivalents and univalents at diakinesis and metaphase-I (Plate-7; Figs, 5,6) (Table-58). It can be seen from the table that at Metaphase-I, ring bivalents ranged from 2-11 with 8.67 and rod bivalents with 0.98 per cell. Other than bivalents, quadrivalents, trivalents (Plate-8; Fig. 17) and univalents were also observed at metaphase-I. Quadrivalents ranged from 0-1 with 0.12 per cell and trivalents ranged from 0-2 with 0.72 per cell. Univalents ranged from 0.17 with 2.26 per cell. The maximum number of 16 univalents (Plate-7; Fig. 8) recorded in 3.6% of PMCs.

Precocious separation of some bivalents were also observed (Plate-7; Fig.7). Chiasma frequency as observed at diakinesis was 17.36 per cell and 1.67 per bivalents (Table-59). At anaphase-I, laggards (Plate-8; Fig.9) were observed in 4.28% of PMC's and in 82.35% PMCs normal separation of chromosomes to the poles was observed (Table-60). At anaphase-II in 3.33% of cells laggard (Plate-8; Fig. 10) were observed and in remaining 96.57% cells equal separation (Table-61). At quartet stage formation of micronuclei (Plate-8; Fig. 13) was observed in 4.04% cells and 7n 95.98% cells regular tetrad formation was observed.

Pollen fertility (Plate-8; Fig. 11) percentage was 50.81 whereas fertile pollen size ranged from 24-45 μ with 38.50 μ mean diameter.

Meiosis in F_2 plant progeny

Meiotic studies in 5 selected F_2 plants are as follows:

Plant No. 1:

At metaphase-I, ring bivalents ranged from 1-11 with 7.77 per cell and rod bivalents ranged from 0-10 with 2.99 per cell (Table-62). A range of 0-2 univalents were noticed with 0.27 per cell chiasma frequency as observed at metaphase-I was 18.53 per cell and 1.72 per bivalent (Table-63). At anaphase-I, three lagging chromosomes were observed in 4.47% cells, while 95.36% cells showed normal chromosome separation to the poles (Table-64). At anaphase-II laggards were observed in 2.46% of PMCs. At sporad stage micronuclei were observed in 2.56% PMCs while in 97.5% cells regular tetrad formation was recorded (Table-65).

Percentage pollen fertility was 76.8, and fertile pollen size ranged from 30 to 45 μ with 35.4 μ mean diameter.

Plant No. 2:

At metaphase-I, quadrivalents, bivalents and univalents were noticed. Ring and rod bivalents ranged from 6-11 and 0-4 with 9.92 and 0.65 per cell respectively (Table-62). Quadrivalents ranged from 0-1 with 0.04 per cell and univalents (Plate-8; Fig. 14) ranged from 0-2 with 0.716 per cell. Chiasma frequency was 20.68 per cell and 1.95 per bivalent (Table-63). At anaphase-I, one lagging chromosome was observed in 3.07% of PMCs while 96.39% cells showed equal separation (Table-64). At anaphase-II, equal separation of chromatids was observed in 98.33% cells and in remaining 1.66% cells laggards were observed (Table-65). At sporad stage regular tetrad formation was observed in 98.66% cells except in 1.33% cells where micronuclei formation was noticed (Table-65).

Percentage pollen fertility was 81.3 and fertile pollen size ranged from 32-45 with 42.6 μ mean diameter.

Plant No. 3:

Ring and rod bivalents ranged from 5-11 and 0-4 with 9.00 and 1.14 per cell, at Metaphase-I of meiotic cell division (Table-62). Univalents ranged from 0-4 with 1.49 per cell. Frequency and mean value of univalents was much reduced as compared to F_1 hybrid. The maximum number of four univalents were noticed in 23.28% of PMCs (Plate-8; Fig. 16). Chiasma frequency was 19.16 per cell and 1.88 per bivalent (Table-63). At anaphase-I three laggards in 2.85% cells, and single chromatid bridge (Plate-8; Fig. 19) in 4.26% cells were

observed, whereas, in remaining 92.30% cells normal separation of chromosomes to the poles was observed (Table-63). At anaphase-II, lagards were observed in 4.61% cells and in 95.35% cells equal separation was noticed. At sporad stage micronuclei were observed in 4.87% cells leaving 95.12% cells for regular tetrad formation. Pollen fertility was 85.2%. Fertile pollen size ranged from 33-42 μ with 37.8 μ mean diameter. (Table-65).

Plant No. 4:

At metaphase-I, ring and rod bivalents (Plate-8; Fig. 15) ranged from 7-11 and 0-4 with 9.67 and 0.97 per cell (Table-62). Univalents ranged from 0-2 with 0.71 per cell. Chiasma frequency as observed at metaphase-I, was 20.31 per cell and 1.90 per bivalent (Table-63). At anaphase-I, one lagging chromosome was noticed in 2.50% cells and remaining 97.50% cells showed equal separation of chromosomes (Table-64). At anaphase-II, equal separation of chromatids was observed in all the cells studied (Table-65). At sporad stage, regular tetrad formation was observed in all the cells studied (Table-65).

Pollen fertility percentage was 93.8 and fertile pollen size ranged from 30-42 μ with 39.6 μ mean diameter.

Plant No. 5:

Meiosis in this plant followed normal pattern as bivalents (Plate-8; Fig. 18) were the only chromosomal association at Metaphase-I (Table-62). Ring and rod bivalents ranged from 8-11 and 0-3 with 10.0 and 1.00 per cell respectively. Chiasma frequency was 21.0 per cell and 1.90 per bivalent (Table-63) ^{At anaphase-I} and, anaphase-II (Table-65), equal separation of chromosomes/Chromatids to the poles was observed in all the cells studied. At sporad stage too, regular tetrad formation was observed.

Table - 56

Morphological observations on *Atylosia lineata*. *Atylosia gajanifolia* their F_1 hybrid and F_2 segregants.

| Characters | F_1 | | | | |
|------------------------------------|--|--|-------------------------|--------------|----------------|
| | <u><i>A. lineata</i></u> (♀ parent) | <u><i>A. gajanifolia</i></u> (♂ parent) | (One plant) (12 plants) | | |
| | 1 | 2 | 3 | 4 | 5 |
| Germination | | Hypogeal | Hypogeal | Hypogeal | Hypogeal |
| Shape of first pair of leaves | Ovate | Lanceolate | Lanceolate | Lanceolate | Lanceolate (9) |
| Growth habit | Erect shrub | Erect shrub | Erect shrub | Erect shrub | Erect shrub |
| No. of primary branches | 4 | 5 | 5 | 11 | 9.21 |
| No. of secondary branches | 5 | 95 | 13 | 17 | 12.62 |
| Height of plant (cm) | | | 112 | 185 | 121 |
| Branching angle | Nearly right | Nearly right | Acute | Nearly right | Acute (7) |
| | | | | | N. right (5) |
| Central leaflet: | | | | | |
| shape | Lanceolate | Lanceolate | Lanceolate | Lanceolate | Lanceolate |
| surface | Hairy | Hairy | Hairy | Hairy | Hairy |
| length (cm.) | 5.5 | 5.2 | 5.2 | 8.0 | 8.20 |
| breadth (cm) | 2.5 | 2.4 | 2.4 | 3.2 | 3.41 |
| venation | palm. retic. | palm. retic. | palm. retic. | palm. retic. | palm. retic. |
| length of petiole (cm) | 1.8 | 1.6 | 1.6 | 1.9 | 2.00 |
| leaf apices | Acute | Acute | Acute | Acute | Acute |
| Stem: | | | | | |
| colour | Green | Green | Green | Green | Green |
| woody/soft | Soft | Soft | Soft | Soft | Soft |
| Days from sowing to bud initiation | 101 | 102 | 102 | 145 | 120 |
| Days from sowings to flowering | 124 | 122 | 122 | 165 | 145 |
| Days between bud to flower | 12 | 11 | 11 | 12 | 12 |

Contd....2.

- 2 -

| | 1 | 2 | 3 | 4 | 5 |
|--|--------------------------|----------------------|---|--|---|
| Days between flower to pod | 34 | 32 | 30 | 36 | |
| Flower: | | | | | |
| size of the standard petal (L x B) cm | 1.6x 1.5 Yellow | 1.6 x 1.6 Red | 1.8 x 1.6 Red | 1.6 x 1.6 Yellow (3) Red (9) | |
| colour of the standard petal | Persistent 1.6 | Persistent 1.6 | Persistent 1.5 | Persistent 1.6 | |
| nature of petals | Green | Brown | Brown | Green (6) Brown (2) Green with brown shed (4) | |
| length of style | | | | 3.6 x 1.0 Present Minute (4) prominent (8) | |
| pod: | | | | | |
| colour of pod | Green | Brown | Brown | Green (6) Brown (2) Green with brown shed (4) | |
| pod (L x B) cm | 3.8 x 1.0 | 4.0 x 0.58 | 3.5 x 1.0 | 3.6 x 1.0 | |
| hairs on mature pod | Present Minute | Present prominent | Present interme- diate | Present Minute (4) prominent (8) | |
| beak of pod | 0.42 Shattering | 0.58 Shattering | 0.49 Shattering | 9.48 Shattering (14) Non-shatt. (1) | |
| thickness of pod (cm) | | | | | |
| nature of mature pod | Brown with black dots | Red | Brown with black dots almost missing | Brown with black dots (6) Red (2) light brown (1) dark brown (3) | |
| Seed: | | | | | |
| colour of seed | | | | | |
| thickness of seed | 0.310 | 0.402 | 0.411 | 0.426 | |

Contd....3.

| | 1 | 2 | 3 | 4 | 5 |
|------------------|---|---------|---------|---------|-------------|
| chambers per pod | | 2.05 | 2.90 | 1.90 | 1.95 |
| seed per pod | | 1.7 | 2.8 | 1.3 | 1.50 |
| strophiole | | present | present | present | present |
| days to maturity | | 196 | 195 | 218 | 190 |
| pod set % | | 61.00 | 35.11 | 4.00 | 32.65 |
| ovule fertility | | 84.0 | 92.0 | 30.0 | 45.0 |
| stomata: | | | | | |
| frequency | | 7.0 | 8.0 | 8.0 | 7.5 |
| (L x B) μ | | 12 x 9 | 18 x 15 | 15 x 12 | 15.0 x 12.3 |

(figures in parentheses are the number of F₂ plants)

Table - 57

Observations on somatic chromosome complement of Atylosia lineata (JM 3366) x Atylosia cajanifolia F₁ hybrid

| Ch. No. | Class | Position of constriction | | Length of short arm (u) | Length of long arm (u) | Total chromosome length (u) | L/s arm ratio |
|---------|----------------|--------------------------|--------|-------------------------|------------------------|-----------------------------|---------------|
| | | Prim. | Secon. | | | | |
| 1 | A | SM | SAT | 1.77±0.35 | 2.13 | 3.55 | 1.00 |
| | A ₁ | SM | SAT | 1.42±0.35 | 1.78 | 3.55 | 1.00 |
| 2 | A | SM | | 1.42 | 2.13 | 3.55 | 1.50 |
| | A ₁ | SM | | 1.42 | 2.13 | 3.55 | 1.50 |
| 3 | A | ST | | 1.00 | 2.55 | 3.55 | 2.46 |
| | A ₁ | ST | | 1.02 | 2.51 | 3.53 | 2.46 |
| 4 | A | SM | | 1.42 | 1.77 | 3.19 | 3.19 |
| | B ₁ | M | | 1.42 | 1.42 | 2.84 | 1.00 |
| 5 | B | ST | | 0.71 | 2.13 | 2.84 | 3.00 |
| | B ₁ | SM | SAT | 1.06±0.35 | 1.43 | 2.84 | 1.67 |
| 6 | B | M | | 1.41 | 1.41 | 2.82 | 1.00 |
| | B ₁ | M | | 1.40 | 1.40 | 2.80 | 1.00 |
| 7 | B | SM | | 1.00 | 1.80 | 2.80 | 1.84 |
| | B ₁ | M | | 1.39 | 1.39 | 2.78 | 1.00 |
| 8 | B | SM | | 1.06 | 1.42 | 2.48 | 1.33 |
| | B ₁ | M | | 1.06 | 1.06 | 2.12 | 1.00 |
| 9 | B | SM | | 0.85 | 1.27 | 2.12 | 1.49 |
| | B ₁ | ST | | 0.71 | 1.41 | 2.12 | 2.01 |
| 10 | B | M | | 1.06 | 1.06 | 2.12 | 1.00 |
| | C ₁ | SM | | 0.72 | 1.06 | 1.78 | 1.49 |
| 11 | C | SM | | 0.71 | 1.06 | 1.78 | 1.49 |
| | C ₁ | SM | | 0.71 | 1.06 | 1.78 | 1.49 |

$$T.F \% = \frac{25.77}{60.49} \times 100 = 42.60$$

Karyotypic formula:

$$5A(SM) + 2A(ST) + 6B(M) + 4B(SM) + 2B(ST) + 3C(SM)$$

Table - 58

Chromosome associations at Metaphase - I in Atylosia
lineata x Atylosia cajanifolia (F₁ hybrid)

| No. of cells studied | Chromosome associations at M-I | | | | | frequency per cent | |
|----------------------------|--------------------------------|-------|------------|-----------|------|--------------------|------|
| | IV | III | Ring II | Red II | I | | |
| 83 | 1 | - | 9 | - | - | 1 | 1.20 |
| - | - | 2 | 8 | - | - | 2 | 2.40 |
| - | - | 1 | 9 | - | 1 | 2 | 2.40 |
| - | - | - | 11 | - | - | 18 | 19.8 |
| - | - | - | 10 | 1 | - | 4 | 4.81 |
| - | - | - | 9 | 2 | - | 3 | 3.6 |
| - | - | - | 10 | - | 2 | 9 | 9.9 |
| - | - | - | 9 | 1 | 2 | 2 | 2.40 |
| - | - | - | 8 | 2 | 2 | 4 | 4.81 |
| - | - | - | 7 | 3 | 2 | 5 | 6.0 |
| - | - | - | 6 | 4 | 2 | 4 | 4.81 |
| - | - | - | 5 | 5 | 2 | 1 | 1.20 |
| - | - | - | 6 | 4 | 2 | 3 | 3.6 |
| - | - | - | 7 | 3 | 2 | 2 | 2.40 |
| - | - | - | 9 | - | 4 | 10 | 12.0 |
| - | - | - | 8 | 1 | 4 | 3 | 3.6 |
| - | - | - | 7 | 2 | 4 | 2 | 2.40 |
| - | - | - | 8 | - | 6 | 5 | 6.0 |
| - | - | - | 3 | 0 | 16 | 2 | 2.40 |
| - | - | - | 2 | 1 | 16 | 1 | 1.20 |
| <hr/> | | | | | | | |
| Range | 0-1 | 0-2 | 2-11 | 0-5 | 0-16 | | |
| Mean | 0.012 | 0.072 | 8.67 | 0.98 | 2.26 | | |

Table - 59

Chiasma frequency in Atylosia lineata x Atylosia cajaniifolia F₁ hybrid.

| Plant | Stage | No. of cells studied | Bivalents with 2xma | lxma | No. of univalentes | Total xmeta | xmeta per cell | xmeta per bivalent |
|---|------------|----------------------|---------------------|------|--------------------|-------------|----------------|--------------------|
| <u>A. lineata</u> (♀ parent) | Diakinesis | 50 | 513 | 37 | - | 1063 | 21.26 | 1.93 |
| <u>A. cajaniifolia</u> (♂ parent) | Diakinesis | 50 | 509 | 41 | - | 1059 | 21.18 | 1.92 |
| <u>A. lineata</u> x <u>A. cajaniifolia</u> (F ₁ hybrid) | Diakinesis | 57 | 400 | 190 | 82 | 990 | 17.36 | 1.67 |

Table - 60

Chromosome distribution at anaphase-I of Atylosia lineata x Atylosia cajaniifolia F₁ hybrid

| Plant | No. of cells studied | equal separation | No. of lagging chromosomes | | | | | bridge |
|---|----------------------|------------------|----------------------------|-------------|---|-------------|-------------|--------|
| | | | 1 | 2 | 3 | 4 | 5 | |
| <u>A. lineata</u> (♀ parent) | 80 | 80 (100) | - | - | - | - | - | - |
| <u>A. cajaniifolia</u> (♂ parent) | 75 | 75 (100) | - | - | - | - | - | - |
| <u>A. lineata</u> x <u>A. cajaniifolia</u> (F ₁ hybrid) | 85 | 70 (82.35) | 3 (4.28) | 6 (8.56) | - | 5 (7.14) | 1 (1.42) | - |

(figures in parentheses are per cent)

Table - 51

Chromatid distribution at Anaphase - II in Atylosia lineata x Atylosia cajaniifoliaF₁ hybrid.

| Plant | No. of cells studied | Normal separation | laggards | Quartet stage | | pollen ferti- lity | pollen size | Mean (M) |
|--|----------------------|-------------------|-------------|----------------------|---------------|--------------------|-------------|----------|
| | | | | No. of cells studied | Tetrad | micro- nuclei. | Range (M) | |
| <u>A. lineata</u> (♀ parent) | 75 | 75 (100) | - | 95 | 95 (100) | - | 33 - 42 | 41.4 |
| <u>A. cajaniifolia</u> (♂ parent) | 80 | 80 (100) | - | 85 | 85 (100) | - | 36 - 45 | 43.5 |
| <u>A. lineata</u> x <u>A. cajaniifolia</u> (F ₁ hybrid) | 90 | 87 (96.57) | 3 (3.33) | 98 | 94 (95.98) | 4 (4.04) | 24 - 45 | 33.5 |

(Figures in parentheses are per cent)

Table - 62

Chromosome association at Metaphase - I in Atylosia lineata x Atylosia cajanifolia (F₂ plants)

| Plant No. | No. of cells studied | Chromosome association at | | | | Fre- quency | per cent |
|--------------|----------------------------|---------------------------|------|------|-------|----------------|----------|
| | | M- I | Ring | Rod | | | |
| | | IV | II | II | I | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1 | 102 | - | 11 | - | - | 15 | 14.7 |
| | | - | 10 | 1 | - | 16 | 15.68 |
| | | - | 9 | 2 | - | 12 | 11.76 |
| | | - | 8 | 3 | - | 9 | 8.82 |
| | | - | 7 | 4 | - | 11 | 10.78 |
| | | - | 6 | 5 | - | 8 | 7.84 |
| | | - | 5 | 6 | - | 7 | |
| | | - | 4 | 7 | - | 5 | 4.9 |
| | | - | 3 | 8 | - | 3 | 2.94 |
| | | - | 2 | 9 | - | 1 | 0.98 |
| | | - | 1 | 10 | - | 1 | 0.98 |
| | | - | 6 | 4 | 2 | 4 | 3.92 |
| | | - | 3 | 7 | 2 | 4 | 3.92 |
| | | - | 10 | 0 | 2 | 6 | 5.88 |
| Range | | | 1-11 | 0-10 | 0-2 | | |
| Mean | | | 7.77 | 2.99 | 0.27 | | |
| 2 | 67 | 1 | 8 | - | 2 | 1 | 1.49 |
| | | 1 | 6 | 3 | 2 | 2 | 2.98 |
| | | - | 11 | - | - | 26 | 38.74 |
| | | - | 10 | 1 | - | 8 | 11.92 |
| | | - | 9 | 2 | - | 5 | 7.45 |
| | | - | 8 | 3 | - | 2 | 2.98 |
| | | - | 7 | 4 | - | 2 | 2.98 |
| | | - | 10 | - | 2 | 15 | 22.35 |
| | | - | 9 | 1 | 2 | 6 | 4.47 |
| | | | | | | | |
| Range | | 0-1 | 6-11 | 0-4 | 0-2 | | |
| Mean | | 0.04 | 9.92 | 0.65 | 0.716 | | |

Contd

Contd...2.

- 2 -

| Plant No. | No. of cells studied | Chromosome associations at M-I | | | | Frequency | per cent |
|--------------|----------------------------|-----------------------------------|----|----|---|-----------|----------|
| | | IV | II | II | I | | |
| 3 | 103 | - | 11 | - | - | 21 | 20.38 |
| | | - | 10 | 1 | - | 12 | 11.64 |
| | | - | 9 | 2 | - | 9 | 8.73 |
| | | - | 8 | 3 | - | 8 | 7.76 |
| | | - | 10 | - | 2 | 13 | 12.61 |
| | | - | 9 | 1 | 2 | 7 | 6.79 |
| | | - | 8 | 2 | 2 | 4 | 3.88 |
| | | - | 7 | 3 | 2 | 5 | 4.85 |
| | | - | 9 | 0 | 4 | 8 | 7.76 |
| | | - | 8 | 1 | 4 | 5 | 4.85 |
| | | - | 7 | 2 | 4 | 6 | 5.82 |
| | | - | 6 | 3 | 4 | 3 | 2.91 |
| | | - | 5 | 4 | 4 | 2 | 1.94 |

| | | | |
|-------|------|------|------|
| Range | 5-11 | 0-4 | 0-4 |
| Mean | 9.00 | 1.14 | 1.49 |

| | | | | | | | |
|---|----|---|----|---|---|----|-------|
| 4 | 73 | - | 11 | - | - | 20 | 27.39 |
| | | - | 10 | 1 | - | 12 | 16.32 |
| | | - | 9 | 2 | - | 8 | 21.76 |
| | | - | 8 | 3 | - | 5 | 6.80 |
| | | - | 7 | 4 | - | 2 | 2.72 |
| | | - | 10 | - | 2 | 12 | 16.32 |
| | | - | 9 | 1 | 2 | 8 | 21.76 |
| | | - | 8 | 2 | 2 | 6 | 8.16 |

| | | | |
|-------|------|------|------|
| Range | 7-11 | 0-4 | 0-2 |
| Mean | 9.67 | 0.97 | 0.71 |

| | | | | | | | |
|---|----|---|----|---|---|----|-------|
| 5 | 54 | - | 11 | - | - | 25 | 46.29 |
| | | - | 10 | 1 | - | 12 | 22.2 |
| | | - | 9 | 2 | - | 9 | 16.65 |
| | | - | 8 | 3 | - | 8 | 14.8 |

| | | |
|-------|---------|--------|
| Range | 8-11 | 0-3 |
| Mean | (10.00) | (1.00) |

Table - 63

Chiasma frequency at Metaphase-I in Atylosia lineata x
Atylosia cajaniifolia (F₂ plants)

| Plant No. | No. of cells studied | No. of quadri-valents | Rivalent with | | No. of univa-lents | Total Xmata | Xmata per cell | Xmata per biva-lent |
|-----------|----------------------|-----------------------|---------------|------|--------------------|-------------|----------------|---------------------|
| | | | 2xma | 1xma | | | | |
| 1 | 102 | - | 793 | 305 | 28 | 1891 | 18.53 | 1.72 |
| 2 | 67 | 3 | 665 | 44 | 48 | 1386 | 20.68 | 1.95 |
| 3 | 103 | - | 928 | 118 | 154 | 1974 | 19.16 | 1.88 |
| 4 | 73 | - | 706 | 71 | 52 | 1483 | 20.31 | 1.90 |
| 5 | 54 | - | 540 | 54 | - | 1134 | 21.0 | 1.90 |

Table - 64

Chromosome distribution at Anaphase - I in Atylosia lineata x Atylosia caianifolia (F₂ plants)

| Plant No. | No. of cells studied | Normal separation | No. of lagging chromosomes | | | | Chromatid bridge | |
|-----------|----------------------|-------------------|----------------------------|-------------|-------------|---|------------------|--------|
| | | | 1 | 2 | 3 | 4 | Single | Double |
| 1 | 67 | 64 (95.36) | - | 3 (4.47) | - | - | - | - |
| 2 | 65 | 63 (96.39) | 2 (3.07) | - | - | - | - | - |
| 3 | 70 | 65 (92.30) | - | - | 2 (2.85) | - | 3 (4.26) | - |
| 4 | 80 | 78 (97.50) | 2 (2.50) | - | - | - | - | - |
| 5 | 50 | 50 (100) | - | - | - | - | - | - |

(Figures in parentheses are per cent)

Table - 65

Chromatid distribution at anaphase - II in Atylosia lineata x Atylosia cajanifolia
(\times_2 plants)

| Plant No. | No. of cells studied | No. of Normal separate- tion | Laggards | Bridge | No. of cells studied | Quartet stage | Micro- nuclei. | Pollen ferti- lity | Vertile pollen size Range (n) (n) | Mean (n) |
|-----------|----------------------|---------------------------------|-------------|--------|----------------------|---------------|-------------------|-----------------------|--------------------------------------|----------|
| 1 | 81 | 79 (97.53) | 2 (2.46) | - | 80 | 78 (97.5) | 2 (2.56) | 76.8 | 30-45 | 35.4 |
| 2 | 60 | 59 (98.33) | 1 (1.66) | - | 75 | 74 (98.66) | 1 (1.33) | 81.3 | 33-45 | 42.6 |
| 3 | 65 | 62 (95.35) | 3 (4.61) | - | 82 | 78 (95.12) | 4 (4.87) | 85.2 | 33-42 | 37.8 |
| 4 | 70 | 70 (100) | - | - | 76 | 76 (100) | - | 93.8 | 30-42 | 39.6 |
| 5 | 85 | 85 (100) | - | - | 65 | 65 (100) | - | 91.6 | 30-45 | 37.5 |

(figures in parentheses are per cent)

PLATE - 8

9

12

11

10

13

14

17

15

16

18

19

20

21

500

100

(X 0

100

No.

100

100

100

100

100

100

100

secondary branches respectively. And the F_1 comprised four primary and eight secondary branches.

In both the parents as well as the F_1 hybrid, the stem was green in colour and soft in texture. During first year of growth A. platycarpa exhibited spread of 35 cm and A. mollis, 45 cm. The F_1 hybrid showed spread of 30 cm. The F_1 hybrid was biennial as against A. platycarpa and A. mollis which shows annual and perennial growth habits respectively.

All the F_2 plants studied showed acute angled primary branches along their mainstem. The number of primary and secondary branches ranged from 3 to 9 and 6 to 19 with 6.31 and 8.50 average primary and secondary branches respectively. The spread of F_2 plants ranged from 25 to 56 cm with 38 cm average spread. Stem in all the F_2 's was green in colour and soft in texture.

4. Leaf:

The leaflet shape of A. platycarpa was cuspidate with acute leaf apices and in A. mollis, it was obovate with acute leaf apices. The F_1 hybrid showed intermediate shape of leaf (Plate-9; Fig. 2). Similar to both the parents, F_1 hybrid showed hairy leaf surface. The average length and breadth of central leaflet of F_1 hybrid was 4.40 cm and 3.00 cm, it were 5.16 and 4.16 cm in A. platycarpa and 2.60 cm and 2.46 cm in A. mollis. The average petiolar length was found to be 3.6 cm in A. platycarpa, 1.7 cm in A. mollis and 3.2 cm in F_1 hybrid.

In F_2 plants, six showed cuspidate leaf shape and 4 plants showed ovovate leaf shape. In F_2 's, length and breadth of central leaflet ranged from 2.2 to 5.7 cm; and 2.0 to 5.2 cm respectively. All the F_2 's met with palmately reticulate venation of leaves. All the F_2 's met with hairy

leaf surface and acute leaf apices. Petiolar length ranged from 1.5 to 3.9 cm, the average being 2.8 cm.

6. Days to flowering and maturity:

After sowing, bud initiation took place in 51,80 and 50 days in A. platycarpa, A. mollis and F_1 hybrid respectively. Days to 50% flowering and pod maturity took 60, 90 and 59; and 128, 155 and 140 in A. platycarpa, A. mollis and F_1 hybrid respectively.

On an average, the number of days taken for bud for full development into flower and from pod initiation to pod maturity were 7, 9 and 8; and 27, 37 and 30 in A. platycarpa, A. mollis and F_1 hybrid respectively. Fifty per cent flowering and pod maturity was recorded in 140 days in F_1 , while it were 128 in A. platycarpa and 155 in A. mollis.

In F_2 's, duration for bud initiation ranged from 50 to 93, the average being 58 days and the days from sowing to flowering ranged from 60 to 101, the average being 68 days. For full development of bud into flower, 7 to 10 days were taken and for pod initiation to pod maturity 26 to 38 days. In F_2 's number of days consumed for 50% pod maturity ranged from 120 to 165.

6. Flower:

The colour of standard petal was yellow in both the parents as well as in the F_1 hybrid. In F_1 hybrid size of standard petal was 1.68 cm^2 as against 0.99 cm^2 in A. platycarpa and 2.56 cm^2 in A. mollis (Plate-9; Fig. 3). The nature of standard petal was persistent in both the parents and F_1 hybrid. Stylor length was noticed to be intermediate as it was 1.2 cm in A. platycarpa, 1.6 cm in A. mollis and 1.4 cm in F_1 hybrid. In F_2 's, flower size

ranged from 0.88 to 2.56 cm², the average being 1.68 cm². Similarly the stylon length ranged from 0.9 to 1.6 cm, with 1.4 cm average stylon length. All the F₂ plants were noticed with persistent and yellow colour of standard petals.

7. Pod setting:

Pod setting in the F₁ hybrid was 83.33% as against 74.0% in A. platycarpa and 40.0% in A. mollis (Table-66). In F₂ plants pod setting percentage ranged from 61.2 to 88.0, the average being 82.0% pod setting. Most of the F₂'s showed higher pod setting percentage in comparison to F₁ hybrid.

8. Pod:

Colour of pod was green in both the parents as well as in F₁ hybrid. On an average the pod sizes in seed parent, pollen parent and their F₁ hybrid were 3.85, 3.60 and 4.9 cm² in A. platycarpa, A. mollis and F₁ hybrid respectively. Similar to female parent A. platycarpa, pods were hairy in F₁ hybrid while male parent A. mollis showed non-hairy pods. Average pod thickness of F₁ hybrid was 0.408 cm as against 0.308 cm in A. platycarpa and 0.500 cm in A. mollis. Shattering nature of mature pods and presence of prominent beak on the distal end of the pod were the consistent feature in the parents as well as in the F₁ and F₂.

In the F₂ plant progeny, all the 10 plants were observed with green colour of pods. Out of these, 7 had hairy and 3 had non-hairy pods (Table-66). The pod sizes ranged from 1.28 to 5.72 cm², the average being 3.60 cm². Thickness of mature pods ranged from 0.208 cm to 0.50 cm with 0.311 cm average pod thickness.

9. Ovule fertility:

Percentage fertility of ovule was in the order of 61.5, 94.0 and 95.5 in A. mollis, F₁ hybrid and A. platycarpa.

In F_2 's it ranged from 56.0 to 96.0%, the average being 74.5%.

10. Seed:

Seed colour of A. mollis and the F_1 was reddish brown with black dots while it was light brown with dark brown dots in A. platycarpa. Average seed thickness in female parent, male parent and F_1 hybrid was recorded to be 0.30 cm, 0.40 cm and 0.36 cm respectively. Chambers per pod on an average, was found to be 2.7 in A. platycarpa, 2.81 in A. mollis and 2.7 in F_1 hybrid as against 2.5 in A. platycarpa and 2.10 in A. mollis. Similar to both the parents the F_1 hybrid possessed strophioled seeds.

In F_2 generation, 6 plants showed light brown seed with dark Brown dots, 2 plants red with black dots, and 2 brown with black dots seed coat colour. Seed thickness ranged from 0.25 cm to 0.40 cm, the average being 0.311 cm seed thickness. Chambers and seed per pod ranged from 1-6 and 1-4.8 respectively, with 2.8 average chambers per pod and 2.2 seed per pod in these F_2 s. All the seeds of F_2 plants were strophioled.

11. Stomata:

Stomatal sizes in A. platycarpa, A. mollis and F_1 (Plate-9; Fig. 4) were 108 μ , 180 μ and 111.3 μ respectively. In F_2 's stomatal size ranged from 108 μ to 180 μ with 118.0 μ average stomatal size. F_1 hybrid showed increased frequency of stomata per unit areas as compared to both the parents.

Atylosia platycarpa x Atylosia mollis F_1 hybrid

Cytology

a) Mitosis

The number of somatic chromosomes counted at metaphase-I

100

was $2n = 22$ (Plate-9; Fig. 1). On the basis of total chromosome length, the somatic complement of F_1 hybrid were grouped into 2 classes (Table-67). The classes A and B contributed by A. mollis and A_1 and B_1 by A. platycarpa. In the F_1 , the somatic chromosomes were linearly arranged in pairs as per their length in descending order. The karyotypic description of each chromosome pair is as follows:

Chromosome pair 1:

Both the chromosomes differed from each other in short arm, long arm and total length by 0.07μ , 0.07μ and 0.14μ respectively. These two chromosomes were similar with regard to position of primary constriction.

Chromosome pair 2:

This chromosome pair was similar in position of primary constriction but difference was observed in short, arm, long arm and total length by 0.14μ , 0.14μ and 0.28μ respectively.

Chromosome pair 3:

Difference in these chromosomes, was observed in short arm, long arm and total length by 0.07μ , 0.07μ and 0.14μ respectively. Both the chromosomes possessed similar position of primary constriction.

Chromosome pair 4:

These two chromosomes differed in short arm and long arm length by 0.14μ and 0.14μ respectively. They did not differ with regard to total chromosome length and position of primary constriction.

Chromosome pair 5:

Homomorphic chromosomes formed this pair as they did not differ with regard to short arm, long arm and total chromosome length and position of primary constriction.

Chromosome pair 6:

Both the chromosomes differed in short arm, and long arm length by $0.07\ \mu$ and $0.07\ \mu$ respectively. Both the chromosomes possessed submedian primary constriction.

Chromosome pair 7:

Chromosomes of this pair differed in short arm and long arm length by $0.14\ \mu$ and $0.14\ \mu$ respectively. They had similar position of primary constriction and total chromosome length.

Chromosome pair 8:

Both the chromosomes differed in short arm and long arm length by $0.07\ \mu$ and $0.07\ \mu$ respectively. They did not differ with regard to total chromosome length and position of primary constriction.

Chromosome pair 9:

Chromosomes of this pair possessed similar position of primary constriction but difference was observed in short arm, long arm and total length by $0.14\ \mu$, $0.15\ \mu$ and $0.01\ \mu$ respectively.

Chromosome pair 10:

Both the chromosomes were similar with regard to position of primary constriction and total chromosome length. Dissimilarity was observed in short arm and long arm length by $0.07\ \mu$ and $0.07\ \mu$ respectively.

Chromosome pair 11:

Both the chromosomes of this pair appeared to be homomorphic with regard to position of primary constriction, short arm, long arm and total chromosome length.

Thus, in this hybrid total chromosome length ranged from 2.12 μ to 3.54 μ with 59.15 cumulative length of chromosome complement and 42.55 T.F.%(Table-67).

Meiotic studies in F_1 hybrid of *A. platycarpa* x *A. mollis*.

Meiotic studies in F_1 hybrid revealed regular formation of bivalents at diakinesis as well as at metaphase-I (Table-68). It can be seen from the Table-68 that there was an increase in the frequency of rod bivalents at metaphase-I in the F_1 hybrid. In the F_1 hybrid, at metaphase-I, ring bivalents ranged from 2-11 with 8.51 per cell and rod bivalents ranged from 0-9 with 2.44 per cell. Chiasma frequency at metaphase-I was 19.51 per cell and 1.77 per bivalent (Table-69). At metaphase-I, one heteromorphic bivalent was observed frequently (Plate-9; Fig. 5). At anaphase-I and II both, regular disjunction of chromosomes/ chromatids was observed in all the PMCs studied (Table-70). At sporad stage, too, regular terad formation was observed. In the present study, high percentage of pollen fertility (92.5) was recorded. Fertile pollen size ranged from 15-48 (Plate-9; Fig. 7) with 30.3 μ mean diameter. Such a wide range of fertile pollen size was the distinct feature of F_1 hybrid, whereas it ranged from 36-42 μ in *A. platycarpa* and 33-36 μ in *A. mollis* (Table-70).

Meiosis in F_2 plant progeny

Meiotic studies in 6 selected F_2 plants are as follows:

Plant No. 1:

At metaphase-I ring bivalents (Plate-9; Fig. 10) ranged from 7-11 with 9.91 per cell and rod bivalents ranged from 0-4 with 1.08 per cell (Table-71). Chiasma frequency at metaphase-I was 21.3 per cell and 1.93 per bivalent (Table-72). At anaphase-I and II regular separation was observed in all the cells resulting in regular tetrad formation. Percentage pollen fertility was 92.5, whereas, fertile pollen size ranged from 30-45 μ with 36.6 μ mean diameter.

Plant No. 2:

At metaphase-I, ring and rod bivalents (Plate-9; Fig. 11) ranged from 3-11 and 0-8 with 7.88 and 3.11 per cell respectively (Table-71). Chiasma frequency at metaphase-I was 18.89 per cell and 1.71 per bivalent (Table-72). At anaphase-I and II regular separation was observed in all the cells studied (Table-73). At sporad stage regular tetrad formation was observed pollen fertility was 96.8%. Fertile pollen size ranged from 33-45 μ with 37.2 μ mean diameter (Table-73).

Plant No. 3:

At metaphase-I, ring and rod bivalents (Plate-9; Fig. 12) ranged from 9-11 and 0-2 with 10.48 and 0.51 per cell respectively (Table-71). Chiasma frequency was 21.48 per cell and 1.95 per bivalent (Table-72). At anaphase-I and II, equal chromosomal separation was noticed resulting in regular formation of tetrads. Percentage pollen fertility was 97.1, while fertile pollen size ranged from 27-45 μ with 33.9 μ mean diameter.

Plant No. 4:

At metaphase-I, ring and rod bivalents (Plate-9; Fig. 13) ranged from 7-11 and 0-4 with 9.90 and 1.09 per cell respectively (Table-71). Chiasma frequency at metaphase-I was 20.90 per cell and 1.90 per bivalent (Table-72). At anaphase-I and II, regular chromosomal separation was observed in all the cells studied, resulted in regular tetrad formation. Pollen fertility percentage was 96.2 and fertile pollen size ranged from 24-45 μ with 37.5 μ mean diameter (Table-73).

Plant No. 5:

Ring and rod bivalents ranged from 4-11 and 0-7 with 9.15 and 1.84 per cell respectively at metaphase-I (Table-71). Chiasma frequency at metaphase-I was 20.15 cell and 1.83 per bivalent (Table-72). At anaphase-I and II regular disjunction of bivalents and univalents were recorded in all the cells (Table-73). At sporad stage regular tetrad formation was noticed. Pollen fertility percentage was 97.1 and fertile pollen size ranged from 32-36 μ with 34.8 μ mean diameter (Table-73).

Plant No. 6:

At metaphase-I ring and rod bivalents ranged from 8-11 and 0-3 with 10.30 and 0.69 per cell respectively (Table-71). Chiasma frequency was 21.30 per cell and 1.93 per bivalent (Table-72). At anaphase-I and II normal separation of chromosomes/chromatids was observed in all the cells studied (Table-73). At sporad stage regular tetrad formation was observed. Pollen fertility percentage was 98.2, whereas fertile pollen size ranged from 30-39 μ with 35.4 μ mean diameter (Table-73).

Table - 66

Morphological observations on Atylosia platycarpa, Atylosia mollis, their F_1 and F_2 's.

| Characters | <u>A. platycarpa</u> (O parent) | <u>A. mollis</u> (O parent) | F_1 (One plant) | F_2 (10 plants) |
|-------------------------------|------------------------------------|--------------------------------|----------------------|------------------------------|
| | 2 | 3 | 4 | 5 |
| Germination | Hypogeal | Hypogeal | Hypogeal | Hypogeal |
| Shape of first pair of leaves | Lanceolate | Ovate | Lanceolate | Lanceolate (8) |
| Growth habit | Herbaceous creeper | Twining herb | Twining herb | Twiner (6) creeper (4) |
| Branching | Acute angle | Acute angle | Acute angle | Acute angle |
| No. of primary branches | 4 | 5 | 4 | 6.31 |
| No. of secondary branches | 7 | 9 | 8 | 8.50 |
| Central leaflet: shape | Cuspidate | Obovate | Inter- mediate | Cuspidate (6) Obovate (4) |
| length (cm) | 5.16 | 2.60 | 4.40 | 4.60 |
| breadth (cm) | 4.16 | 2.46 | 3.00 | 3.50 |
| venation | palm. retic. | palm. retic | palm. retic | palm. retic. |
| length of petiole (cm) | 3.6 | 1.7 | 3.2 | 2.8 |
| leaf apices | acute | acute | acute | acute |
| leaf surface | Hairy | Hairy | Hairy | Hairy |

Contd....2.

Table - 67

Observations on somatic chromosome complement of Atylosia platycarpa x Atylosia mollis F₁ hybrid.

| Ch. pair No. | Class | Position of constriction | | Length of short arm (μ) | Length of long arm (μ) | Total chromo- some length (μ) | L/S arm ratio |
|--------------------|----------------|-----------------------------|-------|-------------------------------------|------------------------------------|---|---------------------|
| | | Prim. | Secn. | | | | |
| 1 | A | SM | SAT | 1.06+0.71 | 1.77 | 3.54 | 1.00 |
| | A ₁ | SM | SAT | 0.99+0.71 | 1.70 | 3.40 | 1.00 |
| 2 | A | ST | | 0.99 | 2.27 | 3.26 | 2.29 |
| | B ₁ | ST | | 0.85 | 2.13 | 2.98 | 2.50 |
| 3 | B | M | | 1.49 | 1.49 | 2.98 | 1.00 |
| | B ₁ | M | | 1.42 | 1.42 | 2.84 | 1.00 |
| 4 | B | SM | | 1.06 | 1.77 | 2.83 | 1.67 |
| | B ₁ | SM | | 1.20 | 1.63 | 2.83 | 1.35 |
| 5 | B | ST | | 0.81 | 2.01 | 2.82 | 2.50 |
| | B ₁ | ST | | 0.81 | 2.01 | 2.82 | 2.50 |
| 6 | B | SM | | 1.27 | 1.42 | 2.69 | 1.11 |
| | B ₁ | SM | | 1.20 | 1.49 | 2.69 | 1.24 |
| 7 | B | SM | | 1.13 | 1.56 | 2.69 | 1.38 |
| | B ₁ | SM | | 1.27 | 1.42 | 2.69 | 1.11 |
| 8 | B | M | | 1.27 | 1.27 | 2.54 | 1.00 |
| | B ₁ | M | | 1.20 | 1.20 | 2.54 | 1.00 |
| 9 | B | SM | | 0.85 | 1.42 | 2.27 | 1.67 |
| | B ₁ | SM | | 0.99 | 1.27 | 2.26 | 1.28 |
| 10 | B | SM | | 0.92 | 1.20 | 2.12 | 1.30 |
| | B ₁ | SM | | 0.85 | 1.27 | 2.12 | 1.49 |
| 11 | B | M | | 1.06 | 1.06 | 2.12 | 1.00 |
| | B ₁ | M | | 1.06 | 1.06 | 2.12 | 1.00 |

$$\text{T.F. \%} = \frac{25.17}{59.15} \times 100 = 42.55$$

Karyotypic formula:

$$2A(SM) + 1A(ST) + 6 B(M) + 10B(SM) + 3 B (ST)$$

| | 1 | 2 | 3 | 4 | 5 |
|---|---|------------|------------|------------|---------------------------|
| Stem: | | | | | |
| colour | | Green | Green | Green | Green |
| woody/soft | | soft | soft | soft | soft |
| spread of plant (cm) | | 35 | 45 | 30 | 38 |
| days from sowing to bud initiation | | 51 | 80 | 50 | 58 |
| days from sowing to flowering | | 60 | 90 | 59 | 68 |
| days between bud to flower | | 7 | 9 | 8 | 8 |
| days between pod initiation to maturity | | 27 | 37 | 30 | 32 |
| Flower: | | | | | |
| Size of the standard petal (L x B) cm. | | 1.1 x 0.9 | 1.6 x 1.6 | 1.4 x 1.2 | 1.4 x 1.2 |
| Colour of the standard petal | | Yellow | Yellow | Yellow | Yellow |
| Nature of petals | | persistent | persistent | persistent | persistent |
| Length of style (cm) | | 1.2 | 1.6 | 1.4 | 1.40 |
| Pod: | | | | | |
| colour of pod | | Green | Green | Green | Green |
| pod (L x B) cm. | | 3.5 x 1.1 | 3.6 x 1.0 | 4.9 x 1.0 | 3.6 x 1.0 |
| Hair on mature pod | | present | absent | present | present (7) absent (3) |

Contd...3

| | 1 | 2 | 3 | 4 | 5 |
|----------------------|---|----------------------------------|-------------------------------|-------------------------------|--|
| Beak of pod | | Prominent | Prominent | Prominent | Prominent |
| Thickness of pod | | 0.308 | 0.500 | 0.408 | 0.311 |
| Nature of mature pod | | Shattering | Shattering | Shattering | Shattering |
| Seed: | | | | | |
| Colour of seed | | Light brown with dark brown dots | Reddish brown with black dots | Reddish brown with black dots | Light brown with dark brown dots (6) red with black dots (2) brown with black dots (2) |
| Thickness of seed | | 0.300 | 0.400 | 0.366 | 0.311 |
| Chambers per pod | | 2.7 | 2.81 | 2.7 | 2.8 |
| Seed per pod | | 2.5 | 2.10 | 2.1 | 2.2 |
| Strophiole | | Present | Present | Present | Present |
| Days to maturity | | 128 | 155 | 140 | 135 |
| Pod set % | | 74.0 | 40.0 | 83.33 | 32.00 |
| Ovule fertility | | 95.5 | 61.5 | 94.0 | 74.5 |
| Stomata: | | | | | |
| Frequency | | 8.0 | 7.0 | 12.0 | 11.0 |
| (L x B) μ | | 12 x 9 | 15 x 12 | 12.1 x 9.2 | 12.3 x 9.6 |

143

(Figures in parentheses are the number of P_2 plants)

Table - 68

Chromosome associations at Metaphase - I in Atylosia platycarpa x Atylosia mollis (F_1 hybrid)

| No. of cells studied | Chromosome associations at M - I | | Frequency | Per cent |
|----------------------|----------------------------------|--------|-----------|----------|
| | Ring II | Rod II | | |
| 70 | 11 | - | 20 | 28.57 |
| | 10 | 1 | 15 | 21.3 |
| | 9 | 2 | 11 | 15.62 |
| | 8 | 3 | 5 | 7.1 |
| | 7 | 4 | 4 | 5.68 |
| | 6 | 5 | 1 | 1.42 |
| | 5 | 6 | 5 | 7.1 |
| | 4 | 7 | 3 | 4.26 |
| | 3 | 8 | 4 | 5.68 |
| | 2 | 9 | 2 | 2.84 |
| Range | 2-11 | 0-9 | | |
| Mean | 8.51 | 2.44 | | |

Table - 69

Chiasma frequency in Atylosia platycarpa x Atylosia mollis (F_1 hybrid)

| Plant | Stage | No. of cells studied | Bivalents with | | Total Xmata | Xmata per cell | Xmata per bivalent |
|--|-----------------|----------------------------|----------------|------|----------------|----------------------|--------------------------|
| | | | 2Xmata | 1Xma | | | |
| <u>A. platycarpa</u> | Diaki- nasis | 70 | 750 | 20 | 1520 | 21.7 | 1.97 |
| <u>A. mollis</u> | Diaki- nasis | 50 | 521 | 29 | 1071 | 21.42 | 1.94 |
| <u>A. platycarpa</u> x <u>A. mollis</u> | Diaki- nasis | 70 | 596 | 174 | 1366 | 19.51 | 1.77 |
| (F ₁ hybrid) | | | | | | | |

Table - 70

Chromosome distribution at Anaphase - I and II in Atylosia platycarpa x Atylosia mollis
(F₁ hybrid)

| Plant | Anaphase - I | | Anaphase - II | | Sporad Stage | | Pollen fertility % | Fertile pollen size | |
|---|----------------------|-------------------|----------------------|-------------------|----------------------|--------------|--------------------|---------------------|----------|
| | No. of cells studied | Normal separation | No. of cells studied | Normal separation | No. of cells studied | Tetrad | | Range (n) | Mean (n) |
| <u>A. platycarpa</u> (♀ parent) | 95 | 95 (100) | 80 | 80 (100) | 110 | 110 (110) | 98.61 | 30 - 36 | 33.0 |
| <u>A. mollis</u> (♂ parent) | 45 | 45 (100) | 50 | 50 (100) | 80 | 80 (100) | 98.4 | 33 - 36 | 34.5 |
| <u>A. platycarpa</u> x <u>A. mollis</u> (F ₁ hybrid) | 115 | 115 (100) | 150 | 150 (100) | 200 | 200 (100) | 92.5 | 15 - 48 | 30.3 |

145

(figures in parentheses are per cent)

Table - 71

Chromosome association at Metaphase - I in Atylosia platycarpa x Atylosia mollis (F₂ plants)

| Plant No. | No. of cells studied | Chromosome association at M- I | | Frequency | Per cent |
|-----------|----------------------|--------------------------------|--------|-----------|----------|
| | | Ring II | Rod II | | |
| 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | 72 | 11 | - | 31 | 43.05 |
| | | 10 | 1 | 16 | 22.08 |
| | | 9 | 2 | 15 | 20.7 |
| | | 8 | 3 | 8 | 11.04 |
| | | 7 | 4 | 2 | 2.76 |
| Range | | 7 - 11 | 0 - 4 | | |
| Mean | | 9.91 | 1.08 | | |
| 2 | 59 | 11 | - | 48 | 81.35 |
| | | 10 | 1 | 12 | 20.28 |
| | | 9 | 2 | 6 | 10.14 |
| | | 8 | 3 | 9 | 15.21 |
| | | 7 | 4 | 7 | 11.83 |
| | | 6 | 5 | 5 | 8.45 |
| | | 5 | 6 | 6 | 10.14 |
| | | 4 | 7 | 4 | 6.76 |
| | | 3 | 8 | 2 | 3.38 |
| Range | | 3 - 11 | 0 - 8 | | |
| Mean | | 7.88 | 3.11 | | |
| 3 | 94 | 11 | - | 61 | 64.89 |
| | | 10 | 1 | 18 | 19.08 |
| | | 9 | 2 | 15 | 15.9 |
| Range | | 9 - 11 | 0 - 2 | | |
| Mean | | 10.48 | 0.51 | | |

Contd... 2.

- 2 -

| 1 | 2 | 3 | 4 | 5 | 6 |
|-------|-----|--|--------------------------------------|---|--|
| 4 | 112 | 11 10 9 8 7 | - 1 2 3 4 | 61 25 18 10 8 | 54.46 22.25 16.02 8.9 7.12 |
| Range | | 7-11 | 0-4 | | |
| Mean | | 9.90 | 1.09 | | |
| 5 | 89 | 11 10 9 8 7 6 5 4 | - 1 2 3 4 5 6 7 | 28 21 15 5 9 6 3 2 | 31.46 23.52 16.8 5.6 10.08 6.72 3.3 2.2 |
| Range | | 4-11 | 0-7 | | |
| Mean | | 9.15 | 1.34 | | |
| 6 | 91 | 11 10 9 8 | - 1 2 3 | 52 21 12 6 | 57.14 22.89 13.08 6.54 |
| Range | | 8-11 | 0-3 | | |
| Mean | | 10.30 | 0.69 | | |

Table - 72

Chiasma frequency in Atylosia platycarpa x Atylosia mollis (F₂ plants)

| Plant No. | Stage | No. of cells studied | Rivalents with 2xmata | 1xma | Total xmata | xmata per cell | xmata per bivalent |
|-----------|------------|----------------------|-----------------------|------|-------------|----------------|--------------------|
| 1 | Meta-phase | 72 | 714 | 78 | 1534 | 21.3 | 1.93 |
| 2 | Meta-phase | 59 | 465 | 184 | 1115 | 18.89 | 1.71 |
| 3 | Meta-phase | 94 | 986 | 48 | 2020 | 21.48 | 1.95 |
| 4 | Meta-phase | 112 | 1109 | 123 | 2341 | 20.90 | 1.90 |
| 5 | Meta-phase | 89 | 815 | 164 | 1794 | 20.15 | 1.83 |
| 6 | Meta-phase | 91 | 938 | 63 | 1939 | 21.30 | 1.93 |

148

Table - 73

Chromosome distribution at Anaphase - I and II in Atylosia platycarpa x Atylosia mollis
(F₂ plants)

| Plant No. | Anaphase - I | | Anaphase - II | | Quartet stage | | Pollen fertility % | fertile pollen size | |
|-----------|----------------------|-------------------|----------------------|-------------------|----------------------|--------------|--------------------|---------------------|----------------|
| | No. of cells studied | Normal separation | No. of cells studied | Normal separation | No. of cells studied | Tetrad | | Range (μ) | Mean (μ) |
| 1 | 85 | 85 (100) | 60 | 60 (100) | 85 | 85 (100) | 92.5 | 30 - 45 | 36.6 |
| 2 | 90 | 90 (100) | 74 | 74 (100) | 92 | 92 (100) | 96.8 | 33 - 45 | 37.2 |
| 3 | 75 | 75 (100) | 69 | 69 (100) | 105 | 105 (100) | 97.1 | 27 - 45 | 33.9 |
| 4 | 82 | 82 (100) | 62 | 62 (100) | 115 | 115 (100) | 96.2 | 24 - 45 | 37.5 |
| 5 | 92 | 92 (100) | 81 | 81 (100) | 150 | 150 (100) | 97.1 | 33 - 36 | 34.8 |
| 6 | 68 | 68 (100) | 80 | 80 (100) | 82 | 82 (100) | 98.2 | 30 - 39 | 35.4 |

149:

PLATE - 9 (A. platycarpa x A. mollis)

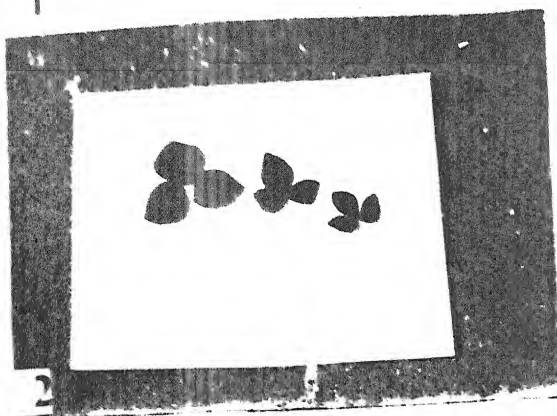
- Fig. 1. Somatic chromosome complement of A. platycarpa x A. mollis F_1 hybrid (X 1500)
- Fig. 2. Leaves of A. platycarpa, F_1 hybrid, and A. mollis (From left to right)
- Fig. 3. Flower of A. platycarpa, F_1 hybrid and A. mollis (From left to right)
- Fig. 4. Stomata of F_1 hybrid showing increased frequency (X 600)
- Fig. 5. 11 bivalents at M-I of F_1 hybrid showing one heteromorphic bivalent (\uparrow) (X 1500)
- Fig. 6. Equal separation of chromosomes at Anaphase of F_1 hybrid (X 1500)
- Fig. 7. Pollen grains of F_1 hybrid (X 1500)
- Fig. 8. Leaves of different F_2 plants.
- Fig. 9. Flower of different F_2 plants.
- Fig. 10. 11 II'_s at Metaphase-I of F_2 hybrid plant No. 1 (X 1500)
- Fig. 11. Eleven bivalents at Metaphase-I of F_2 hybrid plant No. 2 (X 1500)
- Fig. 12. 11 II'_s at Metaphase-I of F_2 hybrid plant No. 3 (X 1500)
- Fig. 13. 11 II'_s at Metaphase-I of F_2 hybrid plant No. 4 (X 1500)

PLATE - 9

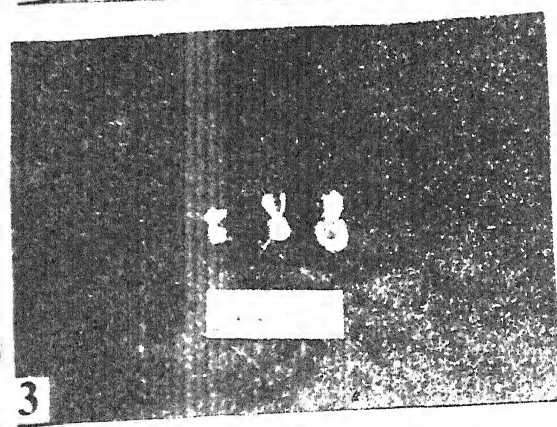
100μ

11 12 13 14 15 16 17 18 19 20

1

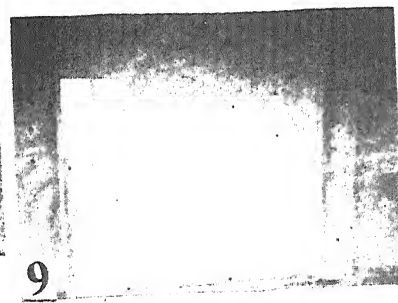


2



3

5



9

6



4



8

10



11



12

13

7

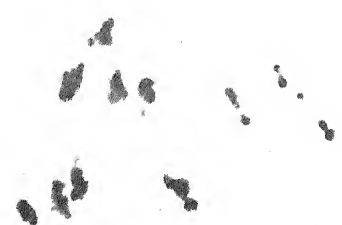
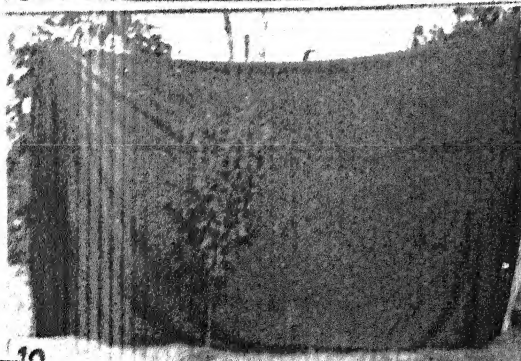
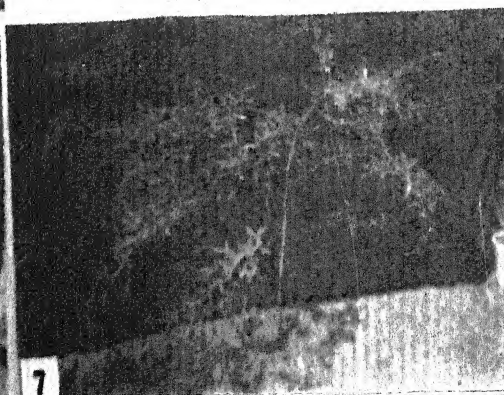
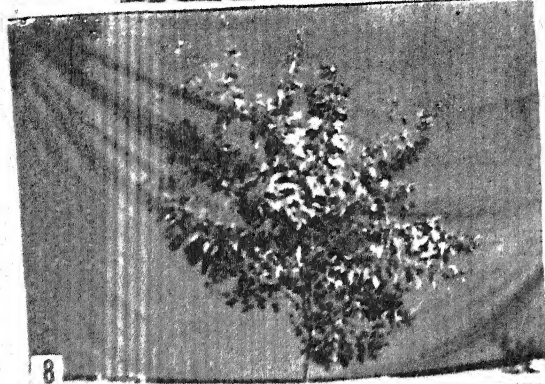
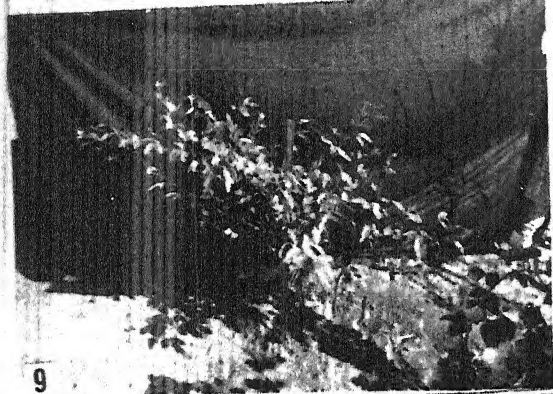
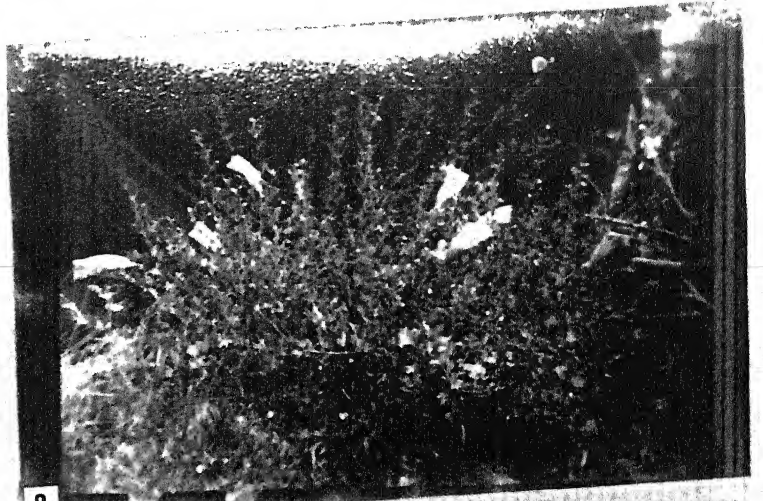
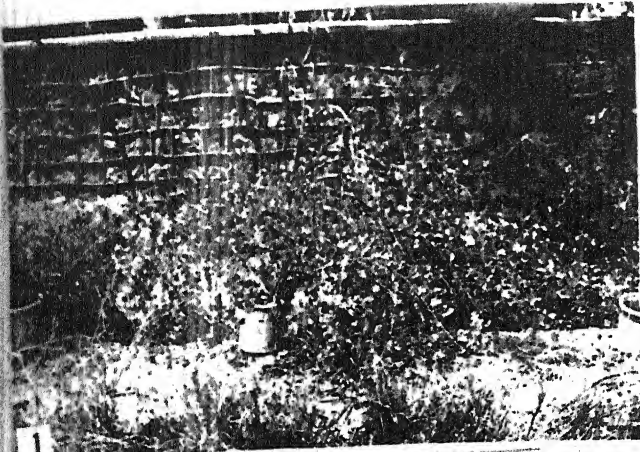


PLATE - 10

- Fig. 1. F_1 hybrid plant of A. albicans x A. cajanifolia
- Fig. 2. F_2 plant of A. albicans x A. cajanifolia showing erect branches.
- Fig. 3. F_2 plant of A. albicans x A. cajanifolia showing semispreading growth habit.
- Fig. 4. F_1 hybrid plant of A. lineata x A. albicans.
- Fig. 5. F_2 plant of A. lineata x A. albicans showing erect growth habit.
- Fig. 6. F_2 plant of A. lineata x A. albicans, showing spreading growth habit.
- Fig. 7. F_2 plant of A. lineata x A. albicans showing erect stem with drooping branches.
- Fig. 8. F_1 hybrid plant of A. lineata x A. cajanifolia
- Fig. 9. F_2 plant of A. lineata x A. cajanifolia showing dwarf and leafy branches.
- Fig. 10. F_2 plant of A. lineata x A. cajanifolia showing tall and erect habit.
- Fig. 11. F_1 hybrid plant of A. platycarpa x A. mollis.

PLATE - 10



STUDIES ON INTERGENERIC HYBRIDS.Atylosia albicans x Cajanus cajanMorphology.

Morphological observations on Atylosia albicans, Cajanus cajan and their hybrids (Table-74) are as follows:

1. Germination and first pair of leaves:

Both the parents, F_1 and F_2 's showed hypogeal germination. The shape of first pair of leaves was ovate in A. albicans and that of Cajanus was lanceolate. The F_1 hybrid exhibited lanceolate shape of first pair of leaves. This indicated dominance of lanceolate shape over the ovate shape.

Out of 10 F_2 plants studied, 8 showed lanceolate shape of first pair of leaves and rest 2 had ovate shape of first pair of leaves.

2. Growth habit:

Atylosia albicans is a twiner and Cajanus cajan is an erect shrub. The cross between twining and erect plants resulted in F_1 hybrid with intermediate growth habit (semierect with drooping branches, (Plate-16; Fig. 9). Out of 10 F_2 's selected for the present study, one was twiner, 3 erect and the rest 6 had semierect growth habit (Plate-16; Fig. 11, 12).

3. Branching angle, stem and height:

Primary branches of Atylosia albicans and Cajanus formed acute angle with their main stem. Likewise, F_1 hybrid also showed acute angled primary branches. At 50%

flowering stage, A. albicans and C. cajan possessed on an average eleven primary branches and seventeen secondary branches; five primary and seventeen secondary branches respectively. And the F_1 hybrid possessed four primary and eight secondary branches. In both the parents as well as in the F_1 hybrid the stem was noticed to be green in colour with soft texture.

In the first year of growth, A. albicans exhibited spread of 87.0 cm and C. cajan showed 103 cm height. The F_1 hybrid grew above the ground upto 15 cm and afterward showed lateral spread of 81.0 cm. All the F_2 segregants exhibited acute angled primary branches. The number of primary branches ranged from 2 to 12, the average being 6.50 and the number of secondary branches ranged from 3 to 18 the average being 11.2. In erect plants, stem height ranged from 71 to 110 cm. In the twiner, spread was recorded to be 73 cm. Plant height in semierects, ranged from 8 to 42 cm with range of their spread from 65 to 121 cm. It is thus the stem height ranged from 8 to 110 cm with the average height of 42.5 cm and spread ranged from 65 to 121 cm with average spread of 95.0 cm in these F_2 's.

4e Leaf:

The leaflet shape in the case of A. albicans was obovate with oval leaf apices and in C. cajan oval oblong with emarginate apices. The F_1 hybrid showed intermediate shape of leaflets (Plate-11; Fig. 1). Both the parents and the F_1 showed non-hairy leaf surface. The F_1 hybrid came up with vigour in leaf size in contrast to both the parents as evident by the average length and breadth of leaflet being 6.5 cm and 4.3 cm respectively. Whereas, it were 4.2 and 3.2 cm in A. albicans and 4.6 and 2.2 cm in C. cajan. The average petiolar length in A. albicans was found to be 4.0 cm and C. cajan 2.6 cm, while it registered 3.8 cm in

the F_1 , indicating thereby that the F_1 was nearer to female parent with regard to this character.

In F_2 generation contrasting characters of leaf shape segregated. 2 Plants had obovate, 2 with oval-oblong and 6 were shown to have intermediate leaf shape. In addition to trifoliate leaves, unifoliate, bifoliate and quadrifoliate leaves were also observed (Plate-12; Fig. 24). All the F_2 plants showed non-hairy leaf surface. Leaf apices as oval, emerginate and intermediate types and leaf venation as palmately reticulate were seen in these plants.

5. Days to flowering and maturity:

After sowing, bud initiation took place in 118 days and 90 days in A. albicans and C. cajan respectively, whereas, in F_1 hybrid initiation of bud formation started only 101 days after sowing. It was observed that 50% flowers appeared in 134, 105 and 124 days in A. albicans, C. cajan and F_1 hybrid respectively. On an average, the number of days consumed from bud initiation to flowering and from pod initiation to pod maturity were 11, 13, 15; and 35, 37, 39 in A. albicans, C. cajan and the F_1 hybrid respectively. Days to 50 per cent pod maturity took 230, 175 and 195 in A. albicans, C. cajan and F_1 respectively. Duration for pod initiation ranged from 101 to 115 days in F_2 's. The days from sowing to 50% flowering ranged from 120 to 148 days. For full development of bud to flower, 11 to 15 days were taken and for pod initiation to pod maturity 35 to 42 days. Fifty per cent pod maturity period ranged from 187 to 212 days in F_2 's in the present study.

6. Flower:

The colour of standard petal was brownish yellow in A. albicans and pale yellow in C. cajan. The F_1 hybrid

showed brownish yellow standard petal (Plate-11; Fig. 2). In F_1 hybrid size of standard petal was 2.98 cm^2 as against 2.56 cm^2 in A. albicans and 2.10 cm^2 in C. cajan (Table-74). The nature of the standard petal was persistent in A. albicans and F_1 hybrid, while it was deciduous in C. cajan. Out of 10 F_2 plants, 6 had brownish yellow standard petal and four showed pale yellow colour. Size of the standard petal ranged from 2.25 to 2.89 cm^2 with the average of 2.40 cm^2 . Nine plants comprised persistent and one plant deciduous standard petal.

7. Pod setting:

Pod setting in F_1 hybrid was 15.0 per cent as against 61.5 per cent in A. albicans and 26.85 per cent in Cajanus cajan (Table-74). In F_2 segregants, pod setting percentage ranged from 11.2 to 30.0, the average being 18.25 per cent. Some of the F_2 's set with more pod setting percentage in comparison to F_1 hybrid.

8. Pod:

Colour of pod in A. albicans was green and in C. cajan, green with black streaks. Pod colour in F_1 hybrid was uniformly brown. On an average the pod sizes in seed parent, pollen parent and their F_1 hybrid were 1.52 cm^2 , 3.78 cm^2 and 4.50 cm^2 respectively. Non-hairy pods with almost similar shape were noticed in the seed parent and the F_1 hybrid (Plate-11; Fig. 3). Average pod thickness of F_1 hybrid was 0.52 cm as against 0.35 cm in A. albicans and 0.70 cm in C. cajan. Similar to seed parent, F_1 hybrid showed shattering nature of mature pods, while C. cajan (Pollen parent) showed non-shattering pods.

In 10 F_2 plants, one with brown, three with green and the rest 6 had green associated with black streaked pods.

The pod size ranged from 1.50 cm^2 to 4.50 cm^2 , the average being 3.15 cm^2 . Prominent beak on the mature pods and absence of hairs was consistent feature observed in all the F_2 progenies. Mature pods with shattering nature was observed in seven F_2 plants and non shattering nature in three F_2 .

9. Ovule fertility:

Percentage fertility of ovule was in the order of 44.8, 72.0 and 85.0 in F_1 , A. albicans and C. cajan. In F_2 's, it ranged from 38.6 to 78.6 and the average being 51.55 per cent.

10. Seed:

The seed colour in female parent was grey with black dots and in pollen parent it was brown. F_1 had the similar seed colour as of the female parent. Average seed thickness in A. albicans was 0.28 cm and in C. cajan 0.70 cm while as intermediate seed thickness 0.502 cm was recorded in F_1 hybrid. Chambers per pod on an average was found to be 2.70 in A. albicans, 2.9 in C. cajan and 2.8 in F_1 hybrid. The average number of seeds per pod was 1.5 in the F_1 hybrid as against 2.10 in A. albicans and 2.2 in C. cajan. Similar to seed parent, F_1 hybrid possessed seed with prominent strophiole, whereas, such character was altogether absent in C. cajan.

In F_2 generation, variety of seed coat colours were observed viz., grey with black dots, brown and brown with black dots (Table-74). The seed thickness ranged from 0.25 cm to 0.70 cm. Strophioled seeds were obtained in 9 and non-strophioled seeds in one F_2 plants.

Stomata

No marked difference in the stomatal frequency between the F_1 and the parents was noticed. However, it varied in size, as 108 μ , 189 μ and 216 μ in female parent, F_1 hybrid and male parent respectively. In F_2 plants, stomatal size ranged from 108 μ to 370 μ with 266.6 μ being average.

Observations on somatic chromosome complement of *Atylosia albicans* x *Cajanus cajan* F_1 hybrid.

Somatic chromosome counts made in the root tip cells of F_1 plant revealed $2n = 22$ (Plate-11; Fig. 4). Unlike the parents (*A. albicans* and *C. cajan*) most of the pairs of mitotic chromosomes were heteromorphic in the F_1 hybrid (Table-75). The karyotypic details are as under.

Chromosome Pair 1:

Both the chromosomes of pair 1 has submedian primary constriction. The chromosomes differ from each other in their short arm length, long arm length and total length by 0.14 μ , 0.35 μ and 0.21 μ respectively.

Chromosome Pair 2:

The chromosomes of this pair differ from each other in their long arm length, and total chromosome length by 0.35 μ and 0.01 μ respectively and one of them was SAT chromosome.

Chromosome Pair 3:

Both the chromosomes of this pair differ in respect of their short and long arm length by 0.36 μ and 0.3 μ respectively. They also differ in position of primary constriction as one chromosome possessed submedian and the other subterminal primary constriction. However, they do not

differ in total length.

Chromosome Pair 4:

This chromosome pair has similar position of primary constriction but dissimilarity exist in their short arm, long arm and total length by $0.22\ \mu$, $0.07\ \mu$ and $0.15\ \mu$ respectively.

Chromosome Pair 5:

Both the chromosomes had similar primary constriction position with the difference from each other in short arm, long arm and total length by $0.30\ \mu$, $0.14\ \mu$ and $0.16\ \mu$ respectively.

Chromosome Pair 6:

Both the chromosomes of this pair appeared to be similar with respect to position of primary constriction, short arm length, long arm length as well as total length.

Chromosome Pair 7:

The chromosomes do not differ in position of their primary constriction and short arm length but difference was observed in their long arm length of $0.02\ \mu$ and total length of $0.02\ \mu$.

Chromosome Pair 8:

Both the chromosomes differ from each other in their short arm and long arm length by $0.10\ \mu$ and $0.10\ \mu$ respectively. These chromosomes showed similarity in their total length as well as the position of primary constriction.

Chromosome Pair 9:

Both the chromosomes are similar with regard to position of primary construction but differ from each other

with respect to short arm, long arm and total length by 0.02 μ and 0.04 μ respectively.

Chromosome Pair 10:

With respect to short and long arm length, this pair of chromosomes showed difference of 0.35 μ and 0.35 μ respectively. These two chromosomes also differ with regard to position of primary constriction as one chromosome has median and the other submedian primary constriction.

Chromosome Pair 11:

This pair do not differ in position of their primary constriction but variation in their short arm length, long arm length and total length by 0.05 μ , 0.05 μ and 0.10 μ respectively was observed.

The total length of the chromosome complement of F_1 hybrid was 63.62 μ , which was intermediate with regard to the length of chromosomal complements of its parents. The total chromosome length varied from 1.98 μ to 3.90 μ with 40.93 per cent T.F.

Meiotic studies in F_1 hybrid of *A. albicans* x *C. cajan*.

Meiotic studies in F_1 hybrid revealed frequent formation of bivalents at diakinesis and metaphase-I. It can be seen from the table-76, that at metaphase-I ring bivalents ranged from 3-11 with 8.52 per cell and formation of rod bivalents ranged from 0-8 with 2.03 per cell. Presence of three heteromorphic bivalents were noticed frequently (Plate-11; Fig. 6). Univalents ranged from 0-4 with 0.47 univalents per cell. Occurrence of loose pairing in some of the bivalents, both at diakinesis as well as metaphase-I were noticed frequently (Plate-11; Fig. 5). Complete pairing of chromosomes at metaphase-I was observed in 78.7%

PMCs and maximum number of 4 unpaired chromosomes were recorded in 1.4% PMCs.

Chiasma frequency as can be seen from the table-77 was 17.2 chiasma per cell and 1.62 per bivalent (Table-77).

At anaphase-I, normal separation of 11:11 chromosomes was observed in majority of the cell (Table-78). 2.85% PMCs were shown to have two lagging chromosomes. At this stage, formation of single chromatid bridge (Plate-11; Fig. 7) was also one of the observed abnormality recorded in 1.42% of PMCs.

At anaphase-II, non-disjunction of chromatids (Plate-11; Figs. 8,10) was observed in 3.75% of PMCs and single chromatid bridge (Plate-11; Fig. 11) in 1.25 % of PMCs. Rest of the PMCs scored exhibited normal separation of chromatids to the poles (Table-79). At sporad stage, monad, dyad, traid and tetrads (Plate-11; Fig. 12) were seen in 2.10 %, 8.42%; 1.05 and 89.25% of PMCs respectively. Formation of micronuclei were observed in 3.15% of the PMCs studied.

Pollen stainability (Plate-11; Fig-13) was recorded to be 62.81 per cent. Stainable pollen size ranged from 35-45 μ with 40.5 μ mean pollen diameter.

Meiosis in F_2 plants.

Meiotic studies made in six selected F_2 plants are as follows:

Plant No. 1:

Chromosomal pairing as evidenced by bivalent formation comprised ring and rod bivalents (Table-80). The

ring bivalents ranged from 6-11 with 9.57 per cell at metaphase-I and rod bivalents ranged from 0-3 with 1.07 per cell. Quadrivalents (Plate-12; Fig. 15) ranged from 0-1 with 0.075 per cell and univalents 0-2 with 0.4 per cell. Chiasma frequency as observed at metaphase-I was 20.52 per cell and 1.92 per bivalent (Table-81). At anaphase-I formation of 3 laggards were recorded in 4.0% of PMCs. At anaphase-II non-disjunction of chromatids was observed in 4.2% of PMCs while separation of chromatids to the poles was observed in 95.14% of PMCs. Occurrence of dyads as the product of some abnormality in meiotic cell division was recorded in 2.84% PMCs at sporad stage. Formation of micronuclei was noticed in 1.42% PMCs. Rest of the PMCs showed tetrad formation.

Plant No. 2:

In this plant, chromosomal association restricted to bivalent formation only (Table-80). Ring and rod bivalents ranged from 7-11 and 0-4 with 9.85 and 1.15 per cell respectively. Chiasma frequency (Table-81) as observed at M-I was 20.84 per cell and 1.89 per bivalent. At anaphase-I (Table-82) equal separation of chromosomes was observed regularly in all the PMCs studied. However, at anaphase-II (Table-83) lagging chromatids (Plate-12; Fig. 20) through in 1.53% cell only were observed, and in 98.46% of PMCs normal separation of chromatids to the poles registered. Stainable pollen size ranged from 33-39 μ with 37.5 μ mean diameter and 78.2% pollen stainability.

Plant No. 3:

Paired chromosomes at metaphase-I revealed ring bivalents which ranged from 0-11 with 8.35 per cell and rod bivalents ranged from 0-4 with 2.10, per cell. Univalents

ranged from 0-4 (Plate-12; Fig. 15) with 1.08 per cell. Chiasma frequency was 18.77 per cell and 1.79 per bivalent. At anaphase-I, one lagging chromosome was recorded in 2.22% of PMCs. The majority of the PMCs (97.8%) showed normal separation of chromosomes. However, at anaphase-II, equal separation of chromatids was noticed in all the PMCs studied. At the sporad stage regular tetrad formation was noticed in 98.4% of PMCs besides 1.42% of cells containing micronuclei.

Fertile pollen size ranged from 33-39 μ with 38.1 μ mean diameter and 85.1% pollen fertility.

Plant No. 4:

Meiotic chromosomal pairing revealed other than bivalents, trivalents and quadrivalent associations at metaphase-I (Plate-12; Figs. 18). Ring and rod bivalents ranged from 4-11 and 0-2 with 10.0 and 0.65 per cell respectively. Quadrivalents ranged from 0-1 with 0.09 per cell and trivalents (Plate-12; Fig. 17) ranged from 0-2 with 0.09 per cell. Chiasma frequency was 21.14 and 1.98 per cell and per bivalent respectively. At anaphase-I one lagging chromosome was observed in 4.0% of PMCs. In 96.0% PMCs, equal separation of chromosomes to the poles was observed (Plate-12; Fig. 19) and Table-82). At anaphase-II non disjunction of chromatids was observed in 2.5% of PMCs through, 1.35% of PMCs exhibited dyad formation at the end of second meiotic division, yet as a result of normal meiosis, majority of cells (96.25%) met with tetrad formation.

Fertile pollen size ranged from 33-42 μ with 12.8 μ mean diameter and 73.7% pollen fertility.

Plant No. 5:

Meiotic chromosomes showed formation of bivalents (Plate-12; Fig. 1) and univalents at metaphase-I. Whereas, ring bivalents ranged from 8-11 with 9.69 per cell and rod bivalents ranged from 0-3 with 0.97 per cell. Univalents ranged from 0-2 with 0.38 per cell. Chiasma frequency was 20.35 per cell and 1.90 per bivalent. At anaphase-I equal separation of chromosomes and at anaphase-II equal separation of chromatids to the poles was observed in all the PMCs studied. At the sporad stage regular tetrad formation was observed in 98.0% PMCs except 2.0% PMCs, where formation of one micronuclei was recorded.

Fertile pollen size ranged from 36-42 μ with 39.3 μ mean diameter and 92.5% pollen fertility.

Plant No. 6:

Meiosis revealed only bivalent association of chromosomes in this plant (Table-80). Ring and rod bivalents ranged from 8-11 and 0-3 with 10.28 and 0.72 per cell respectively. Chiasma frequency was 21.27 per cell and 1.93 per bivalent (Table-81). At anaphase-I and II, both, equal separation was observed in all the PMCs studied. At sporad stage formation of tetrad was observed in all the cells.

Fertile pollen (Plate-12; Fig. 22) size ranged from 36-45 μ with 42.0 μ mean diameter and 76.8% pollen fertility (Table-83).

Table - 74

Morphological observations on Atylosia albicans, Cajanus cajan, their F_1 hybrid and F_2 segregants.

| Characters | <u>A. albicans</u> (♀ parent) | <u>C. cajan</u> (♂ parent) | F_1 (One plant) | F_2 's (10 plants) |
|-------------------------------|----------------------------------|-------------------------------|------------------------|--|
| Germination | Hypogeal | Hypogeal | Hypogeal | Hypogeal |
| Shape of first pair of leaves | Ovate | Lanceolate | Lanceolate | Ovate - 2 Lanceolate - 8 |
| Growth habit | Twining shrub | Erect shrub | Semi-erect | Twining - 1 Erect - 3 Semi-erect - 6 |
| Branching | Acute angled 11 17 | Acute angled 5 17 | Acute angled 4 8 | Acute angled 6.50 11.2 |
| No. of primary branches | | | | |
| No. of secondary branches | | | | |
| Nature of stipules | Persistent | Pugacious | Persistent | Persistent - 8 Pugacious - 2 |
| Stem: | | | | |
| colour | Green | Green | Green | Green |
| woody/soft | Soft | Soft | Soft | Soft |
| Central leaflet: | | | | |
| shape: | Obovate | Oval-oblong | Intermediate | Obovate - 2 Ovaloblong - 2 |
| surface | Non-hairy | Non-hairy | Non-hairy | Intermediate - 6 Non-hairy |
| length (cm) | 4.2 | 4.6 | 6.5 | 7.6 |
| breadth (cm) | 3.3 | 2.0 | 4.3 | 4.4 |
| venation | palm. retic. | palm. retic. | palm. retic. | palm. retic. |

Contd...2.

| | 1 | 2 | 3 | 4 | 5 |
|---|---|--------------------|-----------------------------|--------------------|---|
| length of petiole (cm) leaf apices | | 4.0 Oval | 2.6 Emerginate | 3.8 Oval | 3.2 Oval - 8 Emerginate - 2 |
| Days from sowing to bud initiation | | 118 | 90 | 101 | 120 |
| Days from sowing to flowering | | 134 | 105 | 124 | 142 |
| Days between bud to flower | | 11 | 13 | 15 | 12 |
| Day between pod initiation to maturation | | 35 | 37 | 39 | 36 |
| Flower: | | | | | |
| size of the standard petal (L x B) cm. | | 1.6 x 1.5 | 1.5 x 1.4 | 1.8 x 1.6 | 1.6 x 1.5 |
| colour of the standard petal | | Brownish yellow | pale yellow | Brownish yellow | B. yellow - 6 Pale yellow - 4 |
| nature of petals | | persistent | deciduous | persistent | persistent - 9 deciduous - 1 |
| length of style (cm.) | | 1.6 | 1.5 | 1.7 | 1.6 |
| Pod: | | | | | |
| colour of pod | | Green | Green with black streaks | Brown | Green - 3 Brown - 1 Green with black streaks - 6 |
| pod (L x B) cm. | | 1.9 x 0.8 | 5.4 x 0.7 | 5.0 x 0.9 | 3.5 x 0.9 |
| hairs on mature pod | | Absent | Absent | Absent | Absent |
| beak of pod | | prominent | prominent | prominent | prominent |
| thickness of pod | | 0.35 | 0.75 | 0.52 | 0.48 |

Contd....3.

| 1 | | | | |
|------------------------------------|----------------------|---------------------|----------------------------|--|
| | 2 | 3 | 4 | 5 |
| nature of mature pod | Shattering | Non-shattering | Shattering | Non-shattering |
| Seed: | | | | |
| colour of seed | Grey with black dots | Brown | Grey with black dots | Grey with black dots - 6 Brown - 3 Brown with black dots - 1 |
| thickness of seed (cm) | 0.28 | 0.702 | 0.502 | 0.305 |
| chambers per pod | 2.7 | 2.9 | 2.8 | 2.7 |
| seed per pod | 2.10 | 2.2 | 1.5 | 1.7 |
| strophiole | Present | Absent | Present | Present - 9 Absent - 1 |
| Days to maturity | 230 | 175 | 198 | 205 |
| Pod set % | 61.5 | 26.85 | 15.0 | 18.25 |
| Ovule fertility | 72.0 | 85.0 | 44.8 | 51.55 |
| Seamata frequency (L x B) μ | 9.0 12.0 x 9.0 | 7.00 18.0 x 12.0 | 8.0 18.0 x 10.5 | 7.8 17.2 x 15.5 |
| plant height /spread (cm) | 87.0 | 103 | 81.0 spread 15.0 height | 95.0 spread 42.5 height |

(Figures in parentheses are number of F_2 plants).

Table - 75

Observations on somatic chromosome complement of Atylosia albicans x Cajanus cajan F₁ hybrid

| Chro. No. | Class | Position of constriction | | Length of short arm (μ) | Length of long arm (μ) | Total chromosome length (μ) | L/S arm ratio |
|-----------|-------|--------------------------|--------|-------------------------------|------------------------------|-----------------------------------|---------------|
| | | Prim. | Secon. | | | | |
| 1 | A | SM | | 1.42 | 2.48 | 3.90 | 1.74 |
| 2 | A | SM | | 1.56 | 2.13 | 3.69 | 1.36 |
| 3 | A | SM | | 1.42 | 2.13 | 3.55 | 1.50 |
| 4 | A | SM | SAT | 1.42+0.35 | 1.77 | 3.54 | 1.00 |
| 5 | A | SM | | 1.42 | 2.12 | 3.54 | 1.49 |
| 6 | A | ST | | 1.06 | 2.48 | 3.54 | 2.33 |
| 7 | A | SM | | 1.42 | 1.77 | 3.19 | 1.24 |
| 8 | A | SM | | 1.20 | 1.84 | 3.04 | 1.53 |
| 9 | B | SM | | 1.42 | 1.56 | 2.98 | 1.09 |
| 10 | B | SM | | 1.12 | 1.70 | 2.82 | 1.51 |
| 11 | B | ST | | 0.71 | 2.10 | 2.81 | 2.95 |
| 12 | B | ST | | 0.71 | 2.10 | 2.81 | 2.95 |
| 13 | B | SM | | 1.06 | 1.74 | 2.80 | 1.64 |
| 14 | B | SM | | 1.06 | 1.72 | 2.78 | 1.62 |
| 15 | B | SM | | 1.20 | 1.54 | 2.74 | 1.28 |
| 16 | B | SM | | 1.30 | 1.44 | 2.74 | 1.10 |
| 17 | B | M | | 1.20 | 1.20 | 2.40 | 1.00 |
| 18 | B | M | | 1.18 | 1.18 | 2.36 | 1.00 |
| 19 | B | M | | 1.06 | 1.06 | 2.12 | 1.00 |
| 20 | B | SM | | 0.71 | 1.41 | 2.11 | 1.98 |
| 21 | B | M | | 1.04 | 1.04 | 2.08 | 1.00 |
| 22 | C | M | | 0.99 | 0.99 | 1.98 | 1.00 |

$$T.F.\% = \frac{26.04}{63.62} \times 100 = 40.93$$

Karyotypic formula

$$7A(SM) + 1A(ST) + 4 B(M) + 7B(SM) + 2B(ST) + 1C(M)$$

Table - 76

Chromosome associations at Metaphase - I in Atylosia albicans x Cajanus cajan (F_1 hybrid).

| No. of cells studied | Chromosome associations at M-I | | | | No. of cells per each type | Percentage |
|----------------------------|-----------------------------------|------------|-----------|------|--|------------|
| | IV | Ring II | Rod II | I | | |
| 84 | - | 11 | - | - | 31 | 36.90 |
| - | - | 10 | 1 | - | 5 | 5.95 |
| - | - | 9 | 2 | - | 9 | 10.71 |
| - | - | 8 | 3 | - | 6 | 7.14 |
| - | - | 7 | 4 | - | 4 | 4.76 |
| - | - | 6 | 5 | - | 5 | 5.95 |
| - | - | 5 | 6 | - | 2 | 2.38 |
| - | - | 4 | 7 | - | 3 | 3.57 |
| - | - | 3 | 8 | - | 1 | 1.19 |
| - | - | 5 | 5 | 2 | 6 | 7.14 |
| - | - | 6 | 4 | 2 | 5 | 5.95 |
| - | - | 10 | - | 2 | 5 | 5.95 |
| - | - | 6 | 3 | 4 | 2 | 2.38 |
| <hr/> | | | | | | |
| Range | | 3-11 | 0-8 | 0-4 | | |
| Mean | | 8.52 | 2.03 | 0.47 | | |

Table - 77

Chiasma frequency in Atylosia albicans, Cajanus cajan and their F_1 hybrid

| Plant | Stage | No. of cells studied | Rivalents with | | uni-valents | Total xmata | xmata per cell | xmata per bivalent |
|--|------------|----------------------|----------------|-----|-------------|-------------|----------------|--------------------|
| <u>A. albicans</u> | Diakinesis | 50 | 518 | 32 | 0 | 1068 | 21.36 | 1.94 |
| <u>C. cajan</u> | Diakinesis | 50 | 508 | 42 | 0 | 1058 | 21.16 | 1.92 |
| <u>A. albicans</u> x <u>C. cajan</u> (F_1 hybrid) | Diakinesis | 50 | 330 | 200 | 40 | 860 | 17.2 | 1.62 |

Table - 78

Chromosome distribution at Anaphase - I in Atylosia albicans, Cajanus cajan and their F_1 hybrid.

| Plant | No. of cells studied | Normal separation | Laggards | | | | Chromatid bridge | |
|--|----------------------|-------------------|----------|-------------|---|---|------------------|--------|
| | | | 1 | 2 | 3 | 4 | Single | Double |
| <u>A. albicans</u> | 80 | 80 (100) | - | - | - | - | - | - |
| <u>C. cajan</u> | 90 | 90 (100) | - | - | - | - | - | - |
| <u>A. albicans</u> x <u>C. cajan</u> (F_1 hybrid) | 70 | 67 (95.14) | - | 2 (2.85) | - | - | 1 (1.42) | - |

(Figures in parentheses are per cent)

Table - 79

Chromatid distribution at Anaphase-II in Atylosia albicans, Cajanus cajan and their F_1 hybrid

| Plant | No. of cells studied | Normal separation | Non-disjunct. | Bridge | No. of cells studied | Quartet stage | | | | Pollen fertility % | Pollen size | Range (M) | Mean (x) |
|---|----------------------|-------------------|---------------|-------------|----------------------|---------------|-------------|-------------|--------------|--------------------|-------------|-----------|----------|
| | | | | | | Monad | Dyad | Tetrad | Micro-nuclei | | | | |
| <u>A. albicans</u> (Seed parent) | 100 | 100 (100) | - | - | 95 | - | - | - | - | 98.9 | 33-39 | 36.0 | |
| <u>C. cajan</u> (Pollen parent) | 90 | 90 (100) | - | - | 70 | - | - | - | - | 99.2 | 36-45 | 42.0 | |
| <u>A. albicans</u> x <u>C. cajan</u> (F_1 hybrid) | 80 | 76 (95.0) | 3 (3.75) | 1 (1.25) | 95 | 2 (2.10) | 4 (8.42) | 1 (1.05) | 3 (3.15) | 62.81 | 33-45 | 40.5 | |

(Figures in parentheses are per cent)

Table - 80

Chromosome associations at M - I in Atylosia albicans x Cajanus cajan (F₂ plants)

| Plant No. | No. of cells studied | Chromosome associations at M - I | | | | Frequency | Per cent |
|-----------|----------------------|----------------------------------|------|------|------|-----------|----------|
| | | Ring | | Rod | | | |
| | | IV | II | II | I | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1 | 40 | 1 | 6 | 2 | 2 | 2 | 5.0 |
| | | 1 | 7 | 1 | 2 | 1 | 2.5 |
| | | - | 11 | 0 | - | 20 | 50.0 |
| | | - | 10 | 1 | - | 5 | 12.5 |
| | | - | 9 | 2 | - | 3 | 7.5 |
| | | - | 8 | 3 | - | 4 | 10.0 |
| | | - | 7 | 3 | 2 | 5 | 12.5 |
| Range | | 0-1 | 6-11 | 0-3 | 0-2 | | |
| Mean | | 0.075 | 9.57 | 1.07 | 0.4 | | |
| 2 | 44 | - | 11 | 0 | - | 21 | 47.72 |
| | | - | 10 | 1 | - | 8 | 18.16 |
| | | - | 9 | 2 | - | 5 | 11.35 |
| | | - | 8 | 3 | - | 7 | 15.89 |
| | | - | 7 | 4 | - | 3 | 6.81 |
| | | Range | | | 7-11 | 0-4 | |
| Mean | | | 9.85 | 1.15 | | | |
| 3 | 59 | - | 11 | 0 | - | 15 | 18.9 |
| | | - | 10 | 1 | - | 6 | 10.16 |
| | | - | 9 | 2 | - | 11 | 13.86 |
| | | - | 8 | 3 | - | 9 | 11.34 |
| | | - | 7 | 3 | 2 | 4 | 5.04 |
| | | - | 8 | 1 | 4 | 6 | 10.16 |
| | | - | 7 | 2 | 4 | 3 | 5.0 |
| | | - | - | 9 | 4 | 5 | 8.54 |
| Range | | - | 0-11 | 0-9 | 0-4 | | |
| Mean | | - | 8.35 | 2.10 | 1.08 | | |

Contd...2.

- 2 -

| Plant No. | No. of cells studied | IV | III | Ring II | Rod II | I | Frequency | Per cent |
|-----------|----------------------|-------|------|---------|--------|------|-----------|----------|
| 4 | 41 | 1 | - | 9 | - | - | 2 | 4.87 |
| | | 1 | - | 7 | 2 | - | 2 | 4.87 |
| | | - | 2 | 8 | - | - | 1 | 2.43 |
| | | - | 2 | 7 | 1 | - | 1 | 2.42 |
| | | - | - | 11 | - | - | 18 | 43.90 |
| | | - | - | 10 | 1 | - | 12 | 29.04 |
| | | - | - | 9 | 2 | - | 5 | 12.1 |
| Range | | 0-1 | 0-2 | 7-11 | 0-2 | - | | |
| Mean | | 0.09 | 0.09 | 10.0 | 0.65 | | | |
| 5 | 42 | - | - | 11 | - | - | 15 | 35.7 |
| | | | - | 10 | 1 | - | 8 | 19.04 |
| | | | - | 9 | 2 | - | 5 | 11.9 |
| | | | - | 8 | 3 | - | 6 | 14.28 |
| | | | - | 9 | 1 | 2 | 5 | 11.9 |
| | | | - | 8 | 2 | 2 | 3 | 7.14 |
| | | Range | | - | - | 8-11 | 0-3 | 0-2 |
| Mean | | - | - | 9.69 | 0.97 | 0.38 | | |
| 6 | 48 | - | - | 11 | 0 | - | 28 | 58.24 |
| | | - | - | 10 | 1 | - | 5 | 10.4 |
| | | - | - | 9 | 2 | - | 9 | 18.74 |
| | | - | - | 8 | 3 | - | 6 | 12.48 |
| Range | | - | - | 8-11 | 0-3 | - | | |
| Mean | | | | 10.28 | 0.72 | | | |

Table - 81

Chiasma frequency at M - I in Atylosia albicans x Cajanus cajan (F_1 plants)

| Plant No. | No. of cells studied | No. of Quadri-valents | Bivalents with 2xmata | Bivalents with 1xma | No. of univalents | Total chias-mata | Xmata per cell | Xmata per biva-lent | No. of tri-val-ent |
|-----------|----------------------|-----------------------|-----------------------|---------------------|-------------------|------------------|----------------|---------------------|--------------------|
| 1 | 40 | 3 | 383 | 43 | 16 | 821 | 20.52 | 1.92 | - |
| 2 | 44 | - | 433 | 51 | - | 917 | 20.84 | 1.89 | - |
| 3 | 59 | - | 493 | 124 | 64 | 1108 | 18.77 | 1.79 | - |
| 4 | 41 | 4 | 410 | 27 | - | 867 | 21.14 | 1.98 | 4 |
| 5 | 42 | - | 407 | 41 | 16 | 855 | 20.35 | 1.90 | - |
| 6 | 48 | - | 493 | 35 | - | 1021 | 21.27 | 1.93 | - |

Table - 82

Chromosome distribution at Anaphase - I in Atylosia
albicans x Cajanus cajan (F_2 plants)

| Plant No. | No. of cells studied | Normal separa- tion | No. of lagging chromo- somes | | | | Chromatid bridge | |
|--------------|----------------------------|---------------------------|---------------------------------|---|------------|---|---------------------|--------|
| | | | 1 | 2 | 3 | 4 | single | double |
| 1 | 50 | 48 (96.0) | - | - | 2 (4.0) | - | - | - |
| 2 | 40 | 40 (100) | - | - | - | - | - | - |
| 3 | 45 | 44 (97.8) | 1 (2.22) | - | - | - | - | - |
| 4 | 50 | 48 (96.0) | 2 (4.0) | - | - | - | - | - |
| 5 | 55 | 55 (100) | - | - | - | - | - | - |
| 6 | 60 | 60 (100) | - | - | - | - | - | - |

(Figures in parentheses are per cent)

Table - 83

Chromatid distribution at Anaphase - II in Atylosia albicans x Cajanus cajan (F_2 plants)
(figures in parentheses are per cent)

| plant No. | No. of cells studied | Normal separation | Non-disjunction | Laggs. | No. of cells studied | Quartet stage | | Micro-nuclei. | Pollen fertility % | fertile pollen size | |
|-----------|----------------------|-------------------|-----------------|-------------|----------------------|---------------|---------------|---------------|--------------------|---------------------|------|
| | | | | | | dyad. | tetrad. | | | Range | Mean |
| | | | | | | | | | | (n) | (n) |
| 1 | 70 | 67 (95.14) | 3 (4.28) | - | 71 | 2 (2.84) | 68 (96.56) | 1 (1.42) | 65.6 | 36 - 42 | 39.0 |
| 2 | 65 | 64 (98.46) | - | 1 (1.53) | 65 | - | 64 (97.92) | 1 (1.53) | 78.2 | 33 - 39 | 37.5 |
| 3 | 50 | 50 (100) | - | - | 71 | - | 70 (98.4) | 1 (1.42) | 85.1 | 33 - 39 | 38.1 |
| 4 | 80 | 78 (97.5) | 2 (2.5) | - | 80 | 1 (1.25) | 77 (96.25) | 2 (2.5) | 73.7 | 33 - 42 | 38.4 |
| 50 | 60 | 60 (100) | - | - | 50 | - | 49 (98.0) | 1 (2.0) | 92.5 | 36 - 42 | 39.3 |
| 6 | 75 | 75 (100) | - | - | 60 | - | 58 (96.67) | 2 (3.33) | 76.8 | 36 - 45 | 42.0 |

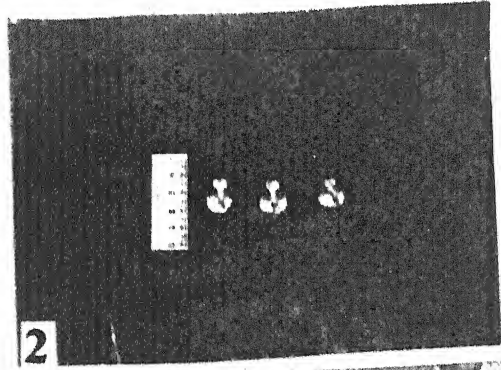
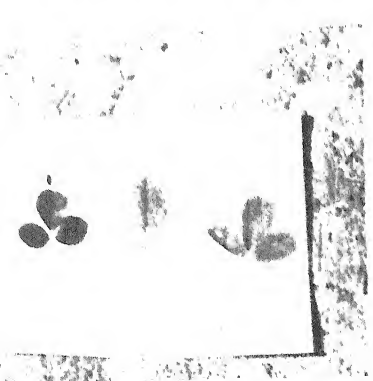
PLATE - 11 (A. albicans x C. cajan)

- Fig. 1. Leaves of A. albicans, F_1 hybrid and C. cajan
(Left to right)
- Fig. 2. Flowers of A. albicans, F_1 hybrid, and C. cajan
(Left to right)
- Fig. 3. Pods of A. albicans, F_1 hybrid and C. cajan
(Left to Right)
- Fig. 4. Somatic chromosome complement of A. albicans x
A. cajanifolia (X 1500)
- Fig. 5. 11 bivalents of F_1 hybrid diakinesis (X1000)
- Fig. 6. 11 bivalents of F_1 hybrid (X1000).
- Fig. 7. Chromatid bridge at Anaphase-I of F_1 hybrid (X1500)
- Fig. 8. Non-disjunction of univalents at Anaphase-II
(X 1500)
- Fig. 9. Scattered chromatids at Anaphase-II of F_1 hybrid
(X 1500)
- Fig. 10. Non-disjunction of chromatids of F_1 hybrid
at Anaphase -II (X 1500)
- Fig. 11. Chromatid bridge at Anaphase-II of F_1 hybrid
(X 1500)
- Fig. 12. Tetrads and dyads at spored stage of F_1 hybrid
(X 400)
- Fig. 13. Pollen grains of F_1 hybrid plant showing partial
sterility (X 160)

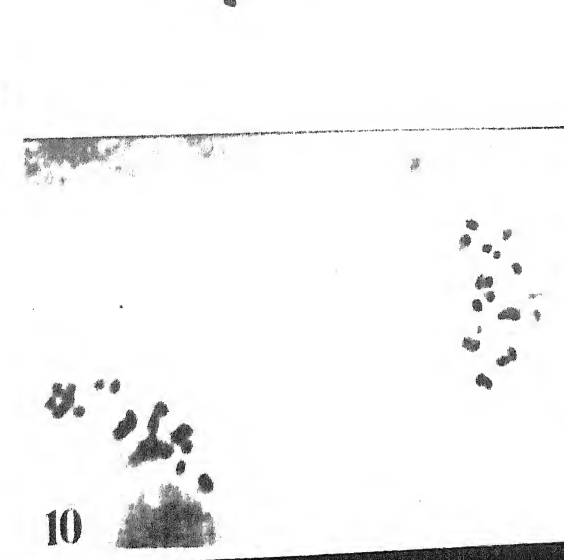
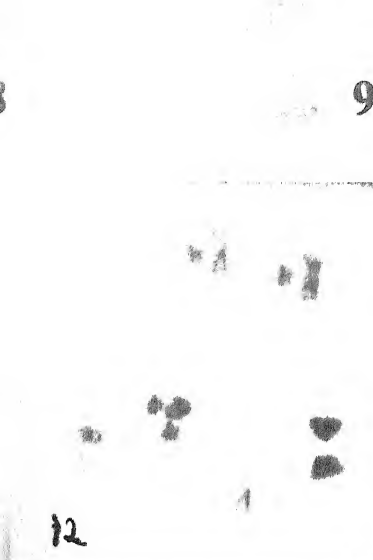
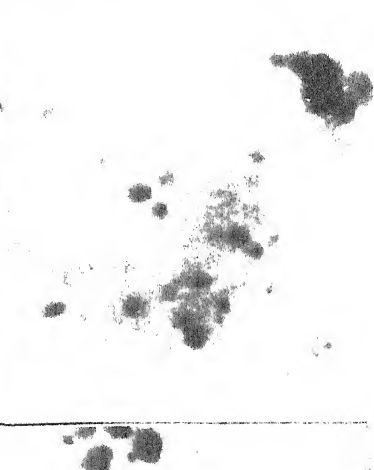
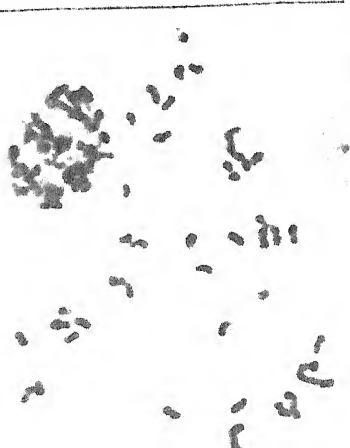
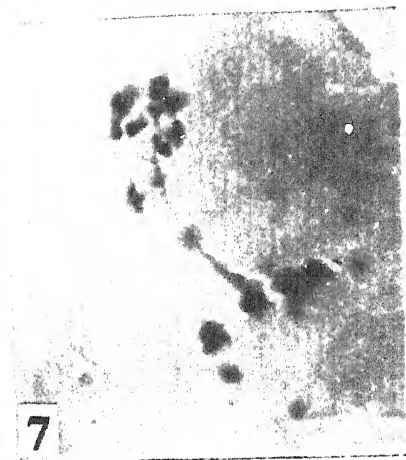
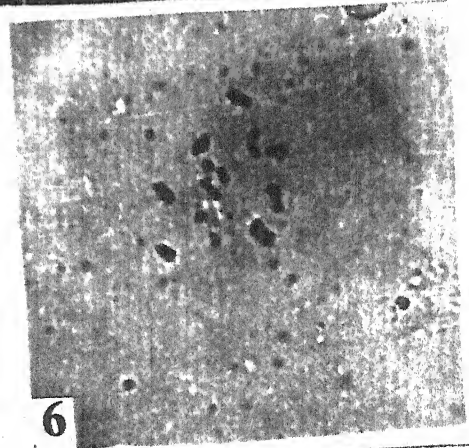
PLATE - II

1000

11 12 13 14 15 16 17 18 19 20



3



12

10

13

PLATE - 12 (A. albicans x C. cajan)

- Fig. 14. 11 bivalents at Metaphase-I of F_2 hybrid plant no. 5 (X 1500) 14
- Fig. 15. 9 II' + 4 I' at Metaphase-I of F_2 hybrid plant No. 3^s (X 1500)
- Fig. 16. 1 IV + 8 II's + 2 I's at Metaphase-I of F_2 hybrid plant No. 2 (X 1500)
- Fig. 17. 2 III' + 8 II's at Metaphase-I of F_2 plant No. 4 (X 1500)
- Fig. 18. 1 IV + 9 II's at Metaphase-I of Plant No. 4 (X 1500)
- Fig. 19. Equal separation of 11-11 Chromosomes at Anaphase-I of plant No. 4 (X 1500) 17
- Fig. 20. Laggards at Anaphase-II of F_2 plant No. 2 (X 1500)
- Fig. 21. Tetrads at sporad stage of Plant No. 4 (X 1000)
- Fig. 22. Pollen grains of F_2 plant No. 6 (X 600)
- Fig. 23. Different types of leaves, flowers and pods of F_2 plants.
- Fig. 24. A single branch of F_2 plant No. 1 showing unifoliate bifoliate and trifoliate leaves and also showing leaves of different shapes on the same branch. 21

PLATE - 12

14



15



16



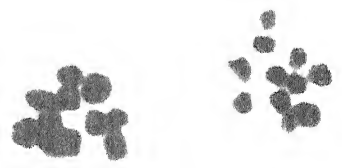
17



18

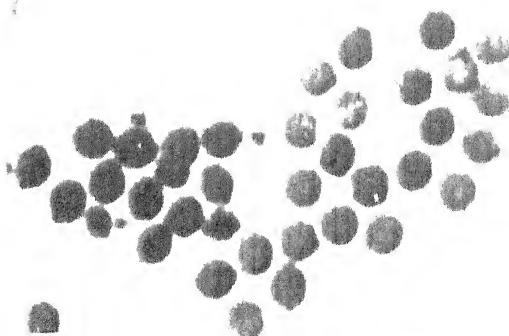


19



23

21



22



20

Atylosia lineata (JM 2639) x Cajanus cajanMorphology

Morphological observations on Atylosia lineata, Cajanus cajan and their hybrids (Table-84) are as follows:

1. Germination and first pair of leaves:

Both the parents, F_1 and F_2 's showed hypogeal germination. The shape of first pair of leaves was ovate in A. lineata and lanceolate in Cajanus cajan. The F_1 hybrid exhibited lanceolate shape of first pair of leaves. This indicated dominance of lanceolate shape over the ovate shape.

Out of 10 F_2 plants studied, 8 showed lanceolate shape of first pair of leaves and rest 2 had ovate shape of first pair of leaves.

2. Growth habit:

Both the parents, F_1 and F_2 's were erect plants. (Plate-16; Fig. 1, 2,3,4).

3. Branching angle, stem and height:

Primary branches of A. lineata formed nearly right angles and that of C. cajan formed acute angles with their main stem. Similar to female parent, F_1 hybrid showed nearly right angles branches. At 50% flowering stage, A. lineata and C. cajan possessed on an average four primary branches and six secondary branches; five primary branches and seventeen secondary branches, respectively. And the F_1 hybrid possessed three primary and six secondary branches. In both the parents as well as in the F_1 hybrid the stem was green in colour with soft texture.

In the first year of growth A. lineata and C. cajan showed an average height of 95 cm and 103 cm, respectively.

In F_2 generation, four plants possessed nearly right angled and six plants acute angled primary branches along the main stem. The number of primary branches ranged from 3 to 12, the average being 5.50 and the number of secondary branches ranged from 9 to 36, the average being 15.50. Plant height ranged from 88 cm to 160 cm with 118 cm average height.

Leaf:

The leaflet shape in case of A. lineata was lanceolate with acute leaf apices and in C. cajan oval oblong with emarginate apices. The F_1 hybrid showed lanceolate shape of leaf with acute apices (Plate-13; Fig.1). Similar to female parent (C. lineata), F_1 hybrid showed hairy leaf surface, while it was non-hairy in C. cajan. The vigour of F_1 hybrid was manifested in leaf size over the parents. The average length and breadth of leaflets of F_1 hybrid was 5.4 cm and 2.3 cm as compared to 5.2 cm and 2.0 cm in A. lineata and 4.6 and 2.0 cm in C. cajan, respectively. The average petiolar length in A. lineata was 2.4 cm and 2.6 cm in C. cajan as compared to 2.4 cm in the F_1 hybrid.

In F_2 plants showed segregation for leaf shape. Two plants had oval oblong and 3 plants were shown to have lanceolate leaf shape. In two segregants, some branches were observed bearing both types of leaves (Plate-13; Fig.13). In F_2 plant progeny, 9 plants were observed with non-hairy leaf surface and one with hairy leaf surface. Leaf apices as acute and emarginate types and leaf venation as palmately reticulate were seen in these plants.

5. Days to flowering and maturity:

after sowing, bud initiation took place in 108 days and 90 days in A. lineata and C. cajanus respectively. whereas, in F_1 hybrid initiation of bud formation started only 110 days after sowing. It was observed that 50% flowers appeared in 124, 105 and 134 days in A. lineata, C. cajan and F_1 hybrid respectively. On an average, the number of days taken for bud initiation to full development into flower and from pod initiation to pod maturity were 13, 13 and 18; and 31, 37 and 41 in A. lineata, C. cajan and the F_1 hybrid respectively. Days to 50% pod maturity were 186, 175 and 210 in A. lineata, C. cajan and F_1 hybrid respectively.

Duration for bud initiation ranged from 95 to 125 days in F_2 s. The days from sowing to 50% flowering ranged from 120 to 158 days. For full development of bud into flower 13 to 16 days were taken and for pod initiation to pod maturity 35 to 40 days. Fifty per cent pod maturation period ranged from 190 to 210 days in F_2 's in the present study.

6. Flower:

The colour of standard petal was yellow, with purple streaks in A. lineata and yellow in C. cajan. The F_1 hybrid showed standard petal colour of yellow with purple streaks (Plate-13; Fig.2). In F_1 hybrid, size of standard petal was 2.72 cm^2 as against 2.1 cm^2 in A. lineata and 2.10 cm^2 in C. cajan. The nature of the standard petal was persistent in A. lineata and deciduous in C. cajan. Similar to female parent (A. lineata) persistent standard petals were observed in F_1 hybrid.

Out of 10 F_2 plants studied, 8 had yellow with purple streaks standard petal, and 2 showed yellow colour of

standard petal. Size of the standard petal ranged from 2.12 to 2.72 cm² with the average of 2.30 cm². Four plants comprised persistent and the 6 plants deciduous standard petal.

7. Pod settings:

Pod setting in F₁ hybrid was 4.42% as against 64.0 in A. lineata and 26.85 in C. cajan (Table-84). In F₂ segregants, pod setting percentage ranged from 9.6 to 34.0 with 18.5% average pod setting. All the F₂'s met with more pod setting percentages in comparison to F₁ hybrid.

8. Pod:

Colour of pod in A. lineata was green and in C. cajan green with black streaks. Pod colour in F₁ hybrid was uniformly dark brown. On an average the pod size in seed parent, pollen parent and their F₁ hybrid were 0.60, 3.78 and 1.00 cm² respectively. Pods of A. lineata was hairy while that of C. cajan non-hairy. Similar to female parent (A. lineata), F₁ hybrid showed hairy pod. F₁ hybrid was nearer to A. lineata in pod shape (Plate-13; Fig. 3). A. lineata showed shattering nature of mature pods while it was non-shattering in C. cajan. F₁ hybrid also showed shattering nature of mature pods indicating dominance of shattering habit of mature pods over non-shattering. Beak of pods of F₁ was intermediate was against prominent in C. cajan and minute in A. lineata. Intermediate thickness of pod was observed in F₁ hybrid being 0.65 cm, while A. lineata and C. cajan showed 0.50 cm and 0.75 cm respectively.

Out of ten F₂ plants, four plants with green, four green with black streaks and 2 with dark brown pods were observed. The average pod size ranged from 0.76 to 3.70 cm² with 1.5 cm². Nine plants met with non-hairy pods and one plant with hairy pods. shattering nature of mature pods was

observed in 8 plants and in the remaining plants non-shattering pods were observed. Four plants with prominent pod beak, four with minute pod beak and 2 with intermediate pod beak were noticed. Thickness of pods in F_2 's ranged from 0.40 to 0.75 cm the average being 0.66 cm.

9. Ovule fertility:

Percentage fertility of ovule was in the order of 32.8, 85.0 and 83.0 in F_1 , C. cajan and A. lineata respectively. In F_2 's it ranged from 30.0 to 62.5% and the average being 48.6%.

10. Seed:

The seed colour in female parent was brown with black dots and in pollen parent it was brown, F_1 had the similar seed colour as of the female parent. Average seed thickness in A. lineata was 0.30 cm and in C. cajan 0.70 cm, while F_1 was nearer to female parent in seed thickness being 0.328 cm. Similar to A. lineata strophiole was present in F_1 hybrid while it was absent in C. cajan. Chambers per pod, on an average was found to be 1.94 in A. lineata, 2.9 in C. cajan and 2.2 in F_1 hybrid. The average number of seeds per pod was 1.82, 2.2 and 0.33 in A. lineata, C. cajan and F_1 hybrid respectively.

In F_2 generation variety of seed coat colour were observed viz., brown with black dots in 3 plants, light brown with dark brown dots in 3 plants, light brown in 2 plants, dark brown in 2 plants. The seed thickness ranged from 0.30 cm to 0.70 cm with an average of 0.41 cm. No. of chambers per pod ranged from 1.5 to 4.0 with 2.3 average and number of seeds per pod ranged from 0.8 to 1.8 with 1.3 seeds per pod. Strophioled seeds were obtained in 9 plants and non-strophioled seeds in one plant.

11. Stomata:

Stomatal size in the seedo parent, pollen parent and F_1 hybrid were 180 μ , 216 μ and 206 μ respectively. In F_2 's stomatal size ranged from 108 μ to 270 μ with 204.3 μ average stomatal size.

Atylosia lineata x Cajanus cajanCytologya) Mitosis:

The number of somatic chromosomes counted at metaphase was $2n = 22$ (Plate-13; Fig. 4). On the basis of total chromosome length, the somatic complement of F_1 hybrid can be grouped into 3 classes (Table-85). The classes A, B and C contributed by Atylosia and A_1 , B_1 and C_1 by Cajanus. In the F_1 , the somatic chromosomes were arranged linearly and the probable homologues are paired off as far as possible. The karyotypic description is as follows:

Chromosome pair 1:

Both the chromosomes of this pair had similar position of primary constriction but differed from each other in short arm, long arm and total length by 0.04 μ , 0.32 μ and 0.02 μ respectively.

Chromosome pair 2:

Both the chromosomes of this pair had similar position of primary constriction but differed from each other in short arm, long arm and total length by 0.06 μ , 0.05 μ and 0.01 μ respectively.

Chromosome pair 3:

Both the chromosomes differ amongst them in short arm, long arm and total length by 0.50 μ , 0.47 μ and 0.03 μ

respectively. They also differ in position of primary constriction as one chromosome possessed submedian and the other sub-terminal primary constriction.

Chromosome pair-4:

The chromosomes of this pair differed amongst them in short arm, long arm and total length by 0.47 μ , 0.43 μ and 0.04 μ respectively. In these two chromosomes differences seen from each other with respect to position of primary constriction and presence of secondary constriction (Table-85).

Chromosome pair 5:

Both the chromosomes had similar position of primary constriction but differed from each other in short arm, long arm and total length by 0.11 μ , 0.09 μ and 0.02 μ respectively.

Chromosome pair 6:

Chromosomes of this pair showed similar position of primary constriction but difference was shown by short arm, long arm and total length as 0.13 μ , 0.15 μ and 0.32 μ respectively.

Chromosome pair 7:

Both the chromosomes differed in long arm length by 0.16 μ and in total length by 0.16 μ while they exhibited similarity in their short arm length and position of primary constriction.

Chromosome pair 8:

These chromosomes possessed similar position of primary constriction and short arm length but difference of 0.08 μ and 0.08 μ was observed in long arm and total length respectively.

Chromosome pair 9:

These two chromosomes are similar with regard to short arm, long arm and total length and position of primary constriction.

Chromosome pair 10:

Both the chromosomes differ in short arm, long arm and total length by 0.39 μ , 0.36 μ and 0.06 μ respectively. They also had different position of primary constriction as one had subterminal and the other submedian primary constriction.

Chromosome pair 11:

These two chromosomes showed similarity in short arm, long arm and total length and position of primary constriction.

Total chromosome length ranged from 7.74 μ to 4.26 μ . The total length of chromosome complement was observed to be 67.77 μ with 39.04% I.F.

Meiotic study in *Atylosia lineata* x *Cajanus cajan* F₁ hybrid:

It can be seen from the table-86 that chromosome pairing as evidenced by bivalent formation revealed ring and rod bivalents at metaphase-I. Ring bivalents ranged from 2-11 with 8.88 per cell and rod bivalents ranged from 0-6 with 2.34 per cell. Univalents and quadrivalent (Plate-13; Fig. 8) ranged from 0-2 and 0-1 with 0.72 and 0.03 per cell respectively. At metaphase-I, occurrence of two heteromorphic bivalents were recorded (Plate-13; Figs. 6 and 7). Chiasma frequency as observed at diakinesis was 17.9 per cell and 1.79 per bivalent (Table-87). At anaphase-I four lagging chromosomes were observed in 1.66% cells and one lagging chromosome in 1.66% cells. In 94.62% cells equal separation of chromosomes (Plate-13; Fig. 10) to the poles was observed.

Double chromatid bridge at anaphase-I (Plate-13; Fig. 9) was observed in 1.66% cells (Table-88). At anaphase-II laggards were noticed in 2.0% and in remaining 98.0% cells equal separation of chromatids was observed. At the sperad stage, regular tetrad formation was observed in 96.6% cells and formation of micronuclei (Plate-13; Fig. 11) were observed in 3.15% cells (Table-89).

Fertile pollen size (Plate-13; Fig. 12) ranged from 35 to 45 μ with 43.5 μ mean diameter and 77.8% pollen fertility.

Meiotic observations in (*C. lineata* x *C. cajan*) F_2 plants.

Meiotic observations in 6 selected F_2 plants are as follows:

Plant No. 1:

Ring bivalents ranged from 4-11 with 10.56 per cell and rod bivalents ranged from 0-5 with 0.34 per cell at metaphase-I (Table-90). Other than bivalents, quadrivalent (Plate-14; Fig. 14) was also observed at metaphase-I with 0-1 range and 0.04 per cell in 4.34% cells. Chiasma frequency was 21.47 per cell and 1.96 per bivalent (Table-91). At anaphase-I, equal separation of chromosomes to the poles was observed (Table-92). At anaphase-II, too, equal separation was observed in all the F_2 cells studied (Table-93). Fertile pollen size ranged from 42 to 45 μ with 42.6 μ mean diameter and 94.6% pollen fertility.

Plant No. 2:

At metaphase-I, ring and rod bivalents ranged from 9-11 and 0-2 with 10.16 and 0.83 per cell respectively. Chiasma frequency was 21.16 per cell and 1.92 per bivalent.

At anaphase-I and II, laggards were seen in 2.1% and 1.42% cells and normal separation was observed in 97.6% and 98.5% cells respectively. At sporad stage, formation of micronuclei was recorded in 1.17% of cells and in remaining 98.8% PMCs regular tetrad formation was observed (Table-93).

Fertile pollen size ranged from 42 to 45 μ with 42.9 μ mean diameter. 91.5% pollen fertility was recorded in this plant.

Plant No. 3:

At metaphase-I ring and rod bivalents ranged from 6-11 and 0-5 with 9.36 and 1.63 per cell. Univalents (Plate-14; Fig. 16) ranged from 0-2 with 0.29 per cell. Chiasma frequency was 19.01 per cell and 1.72 per bivalent. At anaphase-I laggards in 2.5% cells and normal separation of chromosomes in 97.5% cells was observed. At anaphase-II equal separation of chromosomes was observed in all the PMCs studied. At sporad stage tetrads were formed regularly.

Fertile pollen size ranged from 42 to 45 μ with 43.5 μ mean diameter and pollen fertility was 85.2%.

Plant No. 4:

At metaphase-I ring and rod bivalents ranged from 6-11 and 0-4 with 9.21 and 1.45 per cell respectively. Univalent formation ranged from 0-4 with 0.66 per cell. Maximum number of four univalents scored in 9.0% PMCs (Table-90). Chiasma frequency was 19.8 per cell and 1.86 per bivalent (Table-91). At anaphase-I, presence of one laggards was noticed in 2.0% PMCs while 5 lagging chromosomes were seen in 20% cells. At telophase-II, laggards were observed in

4.0% cells and in remaining 96.0% cells normal separation of chromatids was observed. At sporad stage micronuclei were observed in 2.85% cells. Fertile pollen (Plate-14; Fig. 18) size ranged from 42 to 45 μ with 43.8% mean diameter and pollen fertility was 79.6%.

Plant No. 5:

Bivalents (Plate-14; Fig. 15) was the only chromosomal association in this plant. Formation of ring and rod bivalents ranged from 0-11 and 0-11 with 8.86 and 2.13 per cell respectively. Chiasma frequency was 19.8 per cell and 1.86 per bivalent. At anaphase-I, laggards and single chromatid bridge were observed in 2.0% and 11.0% cells respectively. The remaining 94.0% cells showed normal separation of chromosomes. At anaphase-II, equal separation of chromatids to the poles was observed. At sporad stage regular tetrad formation was observed in all the PMCs (Table-93). Fertile pollen size ranged from 42 to 45 μ with 43.2 μ mean diameter and pollen fertility was 81.5%.

Plant No. 6:

In this plant, meiosis follows the normal pattern. At metaphase-I, ring and rod bivalents ranged from 7-11 and 0-4 with 9.08 and 1.62 per cell. Chiasma frequency was 19.78 per cell and 1.84 per bivalent (Table-91). At anaphase-I (Table-92) and at anaphase-II (Table-93) equal separation of chromosomes was observed in all the cells studied. At sporad stage regular formation of tetrads was observed. Fertile pollen size ranged from 42 to 45 μ with 44.1 μ mean diameter and pollen fertility was 87.5%.

Table - 84

Morphological observations on Atylosia lineata. Calanus cajan their F_1 hybrid and F_2 segregants:

| Characters | <u>C. cajan</u> (SNT coll.) (1 Plant) | | | | | <u>F₂'s</u> (10 Plants) |
|--------------------------------------|---|---------------------|--------------|---------------------|--------------------------------------|---------------------------------------|
| | 1 | 2 | 3 | 4 | 5 | |
| Germination | | Hypogeal | Hypogeal | Hypogeal | Hypogeal | |
| Shape of first pair of simple leaves | | Ovate | Lanceolate | Lanceolate | Lanceolate (8) Ovate (2) | |
| Growth habit | | Erect shrub | Erect shrub | Erect shrub | Erect shrub | |
| Branching | | nearly right angled | Acute angled | Nearly right angled | Right angled (4) Acute angled (6) | |
| No. of primary branches | | 4 | 5 | 3 | 5.50 | |
| No. of secondary branches | | 6 | 17 | 6 | 15.50 | |
| Central leaflet: shape | | Lanceolate | oval-or-long | Lanceolate | Lanceol. (8) 57 Ovaloblong (2) | |
| surface | | Hairy | Non-hairy | Hairy | Non-hairy (9) Hairy (1) | |
| length (cm) | | 5.2 | 4.6 | 5.4 | 5.6 | |
| breadth (cm) | | 2.0 | 2.0 | 2.3 | 2.6 | |
| venation | | palm. retic. | palm. retic. | palm. retic. | Palm. retic. | |
| length of petiole (cm) | | 2.4 | 2.6 | 2.8 | 2.7 | |
| leaf apices | | Acute | emarginate | Acute | Acute (8) emarginate (2) | |

Contd...2.

| | 1 | 2 | 3 | 4 | 5 |
|--|---|----------------------------|--------------------------|----------------------------|---|
| Stem: | | | | | |
| colour | | Green | Green | Green | Green |
| woody/soft | | Soft | Soft | Soft | Soft |
| Nature of stipules | | Persistent | Fugacious | Persistent | Persistent(9) Fugacious (1) |
| Days from sowing to bud initiation | | 102 | 90 | 110 | 112 |
| Days from sowing to flowering | | 124 | 105 | 134 | 132 |
| Days between bud to flower (at 50 % flowering) | | 13 | 13 | 18 | 14 |
| Days between flower to pod | | 31 | 37 | 41 | 38 |
| Flower: | | | | | |
| size of the standard petal (L x B) cm. | | 1.5 x 1.4 | 1.5 x 1.4 | 1.7 x 1.6 | 1.6 x 1.5 |
| colour of the standard petal | | yellow with purple streaks | pale yellow | yellow with purple streaks | yellow with purple streaks(8) pale yellow (2) persistent (4) deciduous (6) |
| nature of petals | | Persistent | Deciduous | Persistent | Persistent (4) deciduous (6) |
| length of style (cm) | | 1.5 | 1.5 | 1.6 | 1.6 |
| Pod: | | | | | |
| colour of pod | | Green | Green with black streaks | Dark brown | Green(4) Green streak (4) Dark brown (2) |
| pod (L x B) cm. | | 1.5 x 0.4 | 5.4 x 0.7 | 2.0 x 0.5 | 2.5 x 0.60 |
| hairs on mature pod | | Present | Absent | Present | Present (1) |
| nature of mature pod | | Shattering | Non-shattering | Shattering | Absent (9) Shattering (8) Non-shatt. (2) |

Contd...3.

| | 1 | 2 | 3 | 4 | 5 |
|-------------------------|---|--------------------------|-----------|--------------------------|--|
| beak of pod | | Minute | Prominent | Intermediate | Prominent (4) Minute (4) Intermedi. (2) 0.660 |
| thickness of pod (cm) | | 0.400 | 0.750 | 0.650 | |
| Seeds | | Brown with black dots | Brown | Brown with black dots | Brown with black dots (3) light brown with dark brown dots (3) light brown (2) Dark brown (2) |
| thickness of seed (cm) | | 0.30 | 0.702 | 0.326 | 0.410 |
| no. of chambers per pod | | 1.94 | 2.9 | 2.2 | 2.3 |
| no. of seed per pod | | 1.82 | 2.2 | 0.33 | 1.3 |
| strophiole | | Present | Absent | Present | Present (9) Absent (1) |
| Days to maturity | | 186 | 175 | 210 | 201 |
| Pod set (%) | | 64.0 | 26.85 | 4.42 | 18.5 |
| Ovule fertility (%) | | 83.0 | 85.0 | 32.8 | 43.6 |
| Stomata: | | | | | |
| frequency | | 9.0 | 7.0 | 7.8 | 7.5 |
| (L x R) u | | 15 x 12 | 18 x 12 | 16.5 x 12.5 | 15.6 x 13.1 |
| Height of plant (cm) | | 96 | 103 | 106 | 118 |

(figures in parentheses are No. of F₂ plants)

Table - 85

Observations on somatic chromosome complement of Atylosia lineata (JM 2639) x Cajanus cajan (SNT Coll.) F₁ hybrid

| Ch. No. | Class | Position of constriction | | Length of short arm (μ) | Length of long arm (μ) | Total Chromosome length (μ) | L/S arm ratio |
|---------|----------------|--------------------------|--------|-------------------------|------------------------|-----------------------------|---------------|
| | | Prim. | Secun. | | | | |
| 1 | A | SM | | 1.46 | 2.80 | 4.26 | 1.91 |
| | A ₁ | SM | | 1.42 | 2.82 | 4.24 | 1.98 |
| 2 | A | SM | | 1.38 | 2.28 | 3.56 | 1.65 |
| | A ₁ | SM | | 1.32 | 2.23 | 3.55 | 1.68 |
| 3 | A | SM | | 1.35 | 2.13 | 3.48 | 1.57 |
| | A ₁ | ST | | 0.85 | 2.60 | 3.45 | 3.05 |
| 4 | A | M | SAT | 1.55+0.34 | 1.55 | 3.44 | 0.78 |
| | A ₁ | SM | | 1.42 | 1.98 | 3.40 | 1.39 |
| 5 | A | SM | | 1.53 | 1.84 | 3.37 | 1.20 |
| | A ₁ | SM | | 1.42 | 1.93 | 3.35 | 1.35 |
| 6 | A | M | | 1.66 | 1.66 | 3.32 | 1.00 |
| | A ₁ | M | | 1.50 | 1.50 | 3.00 | 1.00 |
| 7 | B | ST | | 0.71 | 2.13 | 2.84 | 3.00 |
| | B ₁ | ST | | 0.71 | 1.97 | 2.68 | 2.77 |
| 8 | B | SM | | 1.13 | 1.42 | 2.55 | 1.25 |
| | B ₁ | SM | | 1.13 | 1.34 | 2.47 | 1.18 |
| 9 | B | M | | 1.06 | 1.06 | 2.12 | 1.00 |
| | B ₁ | M | | 1.06 | 1.06 | 2.12 | 1.00 |
| 10 | B | ST | | 0.70 | 1.42 | 2.12 | 2.02 |
| | B ₁ | SM | | 1.00 | 1.06 | 2.06 | 1.06 |
| 11 | C | SM | | 0.70 | 1.06 | 1.76 | 1.51 |
| | C ₁ | SM | | 0.70 | 1.04 | 1.74 | 1.46 |

$$T.F. \% = \frac{24.51}{62.77} \times 100 = 39.04$$

Karyotypic formula:

$$3A(M) + 8A(SM) + 1A(ST) + 2B(M) + 3B(SM) + 3B(ST) + 2C(SM)$$

Table - 86

Chromosome associations at Metaphase - I of Atylosia lineata
(JM 2639) x Cajanus cajan (SNT Coll.) F₁ hybrid.

| No. of cells studied | IV | Chromosome associations at M-I | | | | No. of cells per each type | percent- age |
|----------------------------|------|-----------------------------------|------------|-----------|------|-------------------------------------|-----------------|
| | | III | Ring II | Rad II | I | | |
| 94 | 1 | - | 2 | 6 | 2 | 2 | 3.0 |
| | - | - | 11 | - | - | 15 | 22.5 |
| | - | - | 10 | 1 | - | 5 | 7.5 |
| | - | - | 9 | 2 | - | 13 | 19.5 |
| | - | - | 8 | 3 | - | 7 | 10.5 |
| | - | - | 7 | 4 | - | 9 | 13.5 |
| | - | - | 6 | 5 | - | 9 | 13.5 |
| | - | - | 9 | 1 | 2 | 12 | 3.0 |
| | - | - | 10 | - | 2 | 16 | 3.0 |
| | | | 7 | 3 | 2 | 6 | |
| Range | 0-1 | - | 2-11 | 0-6 | 0-2 | | |
| Mean | 0.03 | - | 8.88 | 2.34 | 0.72 | | |

Table - 87

Chiasma frequency in Atylosia lineata, Cajanus cajan, their F_1 hybrid

| Plant | Stage | No. of cells studied | Bivalents with 2xmata | xmata per cell | xmata per bivalent |
|---|------------|----------------------|-----------------------|----------------|--------------------|
| <u>A. lineata</u> (JM 2639) | Diakinesis | 50 | 520 | 21.40 | 1.94 |
| <u>C. cajan</u> (SNT coll.) | Diakinesis | 50 | 508 | 21.16 | 1.92 |
| <u>A. lineata</u> x <u>C. cajan</u> (F_1 hybrid) | Diakinesis | 70 | 554 | 17.9 | 1.79 |

Table - 88

Chromosome distribution at Anaphase - I in Atylosia lineata, Cajanus cajan and their F_1 hybrid.

| Plant | No. of cells studied | Normal separation | Laggards | | | | | Chromatid bridge | |
|---|----------------------|-------------------|----------|----------|---|----------|---|------------------|----------|
| | | | 1 | 2 | 3 | 4 | 5 | single | double |
| <u>A. lineata</u> (♀ parent) | 50 | 50 (100) | - | - | - | - | - | - | - |
| <u>C. cajan</u> (♂ parent) | 60 | 60 (100) | - | - | - | - | - | - | - |
| <u>A. lineata</u> x <u>C. cajan</u> (F_1 hybrid) | 60 | 57 (94.62) | - | 1 (1.66) | - | 1 (1.66) | - | - | 1 (1.66) |

(figures in parentheses are per cent)

Table - 89

Chromatid distribution at Anaphase- II in Atylosia lineata, Cajanus cajan and their F_1 hybrid.

| Plant | Anaphase - II | | | Quartet stage | | Pollen fertility % | Fertile pollen | |
|---|----------------------|-------------------|-------------|----------------------|---------------|--------------------|----------------|----------|
| | No. of cells studied | Normal separation | Bridge lagg | No. of cells studied | Tetrad nuclei | | Range (n) | Mean (n) |
| <u>A. lineata</u> (♀ parent) | 80 | 80 (100) | - | 90 | 90 (100) | 99.7 | 36-42 | 39.0 |
| <u>C. Cajan</u> (♂ parent) | 90 | 90 (100) | - | 85 | 85 (100) | 99.2 | 36-45 | 42.0 |
| <u>A. lineata</u> x <u>C. cajan</u> (F_1 hybrid) | 100 | 98 (98.0) | 2 (2.0) | 95 | 92 (96.6) | 77.8 (3.15) | 33-45 | 43.5 |

101

(Figures in parentheses are per cent)

Table - 90

Chromosome associations at Metaphase - I in Atylosia lineata x Cajanus cajan (F_2 plants).

| Plant No. | No. of cells studied | Chromosome associations at M-I | | | | frequency | Per cent |
|-----------|----------------------|--------------------------------|---------|--------|------|-----------|----------|
| | | IV | Ring II | Rod II | I | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1 | 46 | 1 | 4 | 5 | - | 2 | 4.34 |
| | | - | 11 | 0 | - | 38 | 78.26 |
| | | - | 10 | 1 | - | 6 | 13.02 |
| Range | | 0-1 | 4-11 | 0-5 | | | |
| Mean | | | 10.56 | 0.34 | | | |
| 2 | 36 | - | 11 | 0 | - | 18 | 49.86 |
| | | - | 10 | 1 | - | 6 | 16.6 |
| | | - | 9 | 2 | - | 12 | 33.3 |
| Range | | - | 9-11 | 0-2 | | | |
| Mean | | - | 10.16 | 0.83 | | | |
| 3 | 55 | - | 11 | - | - | 21 | 38.01 |
| | | - | 10 | 1 | - | 15 | 27.27 |
| | | - | 8 | 3 | - | 6 | 10.86 |
| | | - | 7 | 4 | - | 5 | 9.09 |
| | | - | 6 | 5 | 2 | 5 | 9.09 |
| | | - | 7 | 4 | 2 | 3 | 5.43 |
| Range | | | 6-11 | 0-5 | 0-2 | | |
| Mean | | | 9.36 | 1.63 | 0.29 | | |
| 4 | 33 | - | 11 | - | 0 | 15 | 45.45 |
| | | - | 10 | 1 | - | 3 | 9.0 |
| | | - | 9 | 2 | - | 2 | 6.0 |
| | | - | 7 | 4 | - | 5 | 15.1 |
| | | - | 8 | 2 | 2 | 3 | 9.0 |
| | | - | 7 | 3 | 2 | 2 | 6.0 |
| | | - | 6 | 3 | 4 | 3 | 9.0 |
| Range | | | 6-11 | 0-4 | 0-4 | | |
| Mean | | | 9.21 | 1.45 | 0.66 | | |

Contd...2.

- 2 -

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|----|---|----|----|---|----|-------|
| 5 | 45 | - | 11 | - | - | 21 | 46.62 |
| | | - | 10 | 1 | - | 5 | 11.1 |
| | | - | 9 | 2 | - | 6 | 13.32 |
| | | - | 8 | 3 | - | 1 | 2.22 |
| | | - | 7 | 4 | - | 3 | 6.66 |
| | | - | 6 | 5 | - | 2 | 4.44 |
| | | - | 5 | 6 | - | 2 | 4.44 |
| | | - | 4 | 7 | - | 2 | 4.44 |
| | | - | 3 | 8 | - | 1 | 2.22 |
| | | - | 2 | 9 | - | 1 | 2.22 |
| | | - | 0 | 11 | - | 1 | 2.22 |

| | | | |
|-------|---|------|------|
| Range | - | 0-11 | 0-11 |
| Mean | - | 8.86 | 2.13 |

| | | | | | | | |
|---|----|---|----|---|---|----|------|
| 6 | 50 | - | 11 | 0 | - | 15 | 30.0 |
| | | - | 10 | 1 | - | 9 | 18.0 |
| | | - | 9 | 2 | - | 12 | 24.0 |
| | | - | 8 | 3 | - | 8 | 16.0 |
| | | - | 7 | 4 | - | 6 | 12.0 |

| | | | | |
|-------|---|---|-------|------|
| Range | - | - | 7- 11 | 0-4 |
| Mean | - | - | 9.08 | 1.62 |

Table - 91

Chiasma frequency at M-I in Atylosia lineata x Cajanus cajan
(F₂ plants)

| Plant No. | No. of cells studied | Quadri-valent with 4xmata | Bivalents with 2xmata | Bivalents with 1xmata | Uni-val-ents | Total Xmata | Xmata per cell | Xmata per bivalent |
|-----------|----------------------|---------------------------|-----------------------|-----------------------|--------------|-------------|----------------|--------------------|
| 1 | 46 | 1 | 486 | 16 | - | 988 | 21.47 | 1.96 |
| 2 | 36 | - | 366 | 30 | - | 762 | 21.16 | 1.92 |
| 3 | 55 | - | 515 | 90 | 16 | 1046 | 19.01 | 1.72 |
| 4 | 33 | - | 304 | 48 | 22 | 659 | 19.8 | 1.86 |
| 5 | 45 | - | 399 | 96 | - | 894 | 19.8 | 1.80 |
| 6 | 50 | - | 454 | 81 | - | 989 | 19.78 | 1.84 |

Table - 92

Chromosome distribution at Anaphase - I in Atylosia
lineata x Cajanus cajan (F_2 plants)

| Plant No. | No. of cells studied | Normal separa- tion | Laggards | | | | Chromatid bridge | |
|--------------|----------------------------|---------------------------|------------|------------|---|------------|------------------|--------|
| | | | 1 | 2 | 3 | 4 | Single | Double |
| 1 | 55 | 55 (100) | - | - | - | - | - | - |
| 2 | 46 | 45 (97.6) | 1 (2.1) | - | - | - | - | - |
| 3 | 40 | 39 (97.5) | - | 1 (2.5) | - | - | - | - |
| 4 | 50 | 48 (96.0) | 1 (2.0) | - | - | 1 (2.0) | - | - |
| 5 | 50 | 47 (94.0) | 1 (2.0) | - | - | - | (4.0) | - |
| 6 | 65 | 65 (100) | - | - | - | - | - | - |

(Figures in parentheses are per cent)

Table - 93

Chromatid distribution at anaphase - II in Atylosia lineata x Cajanus cajan (F₂ plants)

| Plant No. | No. of Normal cells separation | | Sporad stage | | Pollen fertility % | Fertile pollen size | |
|-----------|--------------------------------|-------------|----------------------|----------------------|--------------------|---------------------|----------------|
| | studied | Laqgs | No. of cells studied | Tetrad Micro-nuclei. | | Range (μ) | Mean (μ) |
| 1 | 60 | - | 75 | 75 (100) | 94.6 | 42 - 45 | 42.6 |
| 2 | 71 | 1 (98.5) | 85 | 84 (98.8) | 91.5 | 42 - 45 | 42.9 |
| 3 | 85 | - | 80 | 80 (100) | 85.2 | 42 - 45 | 43.5 |
| 4 | 50 | 2 (96.0) | 70 | 68 (97.14) | 79.6 | 42 - 45 | 43.8 |
| 5 | 55 | - | 65 | 65 (100) | 81.8 | 42 - 45 | 43.2 |
| 6 | 60 | - | 50 | 50 (100) | 87.5 | 42 - 45 | 44.1 |

(Figures in parentheses are per cent)

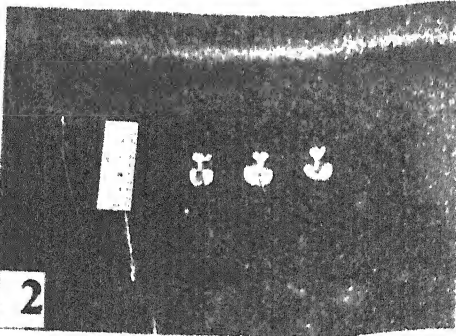
Plate - 13 (A. lineata x C. cajan)

- Fig. 1. Leaves of A. lineata, F_1 hybrid, and Cajanus cajan (Left to Right)
- Fig. 2 . Flowers of A. lineata, F_1 hybrid and C. cajan (Left to Right)
- Fig. 3. Pods of A. lineata, F_1 hybrid and C. cajan. (Left to Right)
- Fig. 4. Somatic chromosome complement of A. lineata x C. cajan (X 1500)
- Fig. 5. 11 bivalents of F_1 hybrid at diakinesis (X 1500)
- Fig. 6&7. 11 bivalents of F_1 hybrid at Metaphase-I showing two heteromorphic bivalents (\uparrow) (X 1500)
- Fig. 8. 1 IV + 8 II's + 2 I's of F_1 hybrid at Metaphase-I (X 1500)
- Fig. 9. Double chromatid bridge at Anaphase-I of F_1 hybrid (X 1500)
- Fig. 10. Equal separation of 11-11 chromosomes at Anaphase-I (X 1500)
- Fig. 11. Micronuclei at spored stage (X 600)
- Fig. 12. Pollen grains of F_1 hybrid showing partial sterility (X 600)
- Fig. 13. A single branch of F_2 plant No. 1 showing leaves like C. cajan, A. lineata and F_1 hybrid plant.

PLATE - 13

5) 11 22 33 44 55 66 77 88 99

4



1

2

3

5

6

7

8

9

11

10

12



PLATE - 14

- Fig. 14. 1 III + 9 II' of F_2 hybrid plant No. 1 of A. lineata x C. cajan at Metaphase-I (X 1500)
- Fig. 15. 11 bivalents of F_2 hybrid plant No. 3 of A. lineata x C. cajan at Metaphase-I (X 1500)
- Fig. 16. 10 II' + 2 I' of F_2 hybrid plant No. 3 of A. lineata x C. cajan, at Metaphase-I (X 1500)
- Fig. 17. One univalent away from the group at Anaphase-I of F_2 hybrid plant No. 3 of A. lineata x C. cajan (X 1500)
- Fig. 18. Pollen grains of F_2 hybrid plant No. 4 showing partial sterility (X 600)
- Fig. 1 to 8 : A. scarabaeoides x C. cajan.
- Fig. 1. Leaves of A. scarabaeoides, F_1 hybrid and C. cajan (Left to Right)
- Fig. 2. Flowers of A. scarab. F_1 hybrid, and C. cajan (Left to Right)
- Fig. 3. Pods of A. scarabaeoides, F_1 hybrid and C. cajan (Left to Right)
- Fig. 4. Somatic chromosome complement of A. scarabaeoides x C. cajan F_1 hybrid (X 1500)
- Fig. 5. 11 bivalents at diakinesis of F_1 hybrid (X 1500)
- Fig. 6. 11 bivalents of F_1 hybrid at Metaphase-I showing one heteromorphic bivalent (\uparrow) (X 1500)
- Fig. 7. 10 II's + 2 I's at Metaphase-I of F_1 hybrid (X 1500)
- Fig. 8. 2 III's + 8 II's at Metaphase-I of F_1 hybrid (X 1500)

PLATE - 14

10

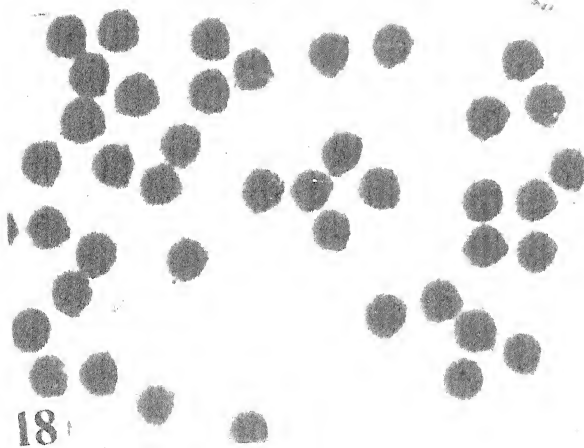
14

15

16

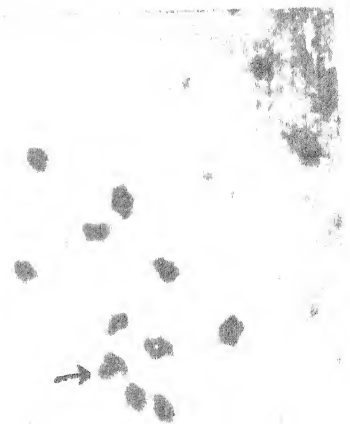


17



18

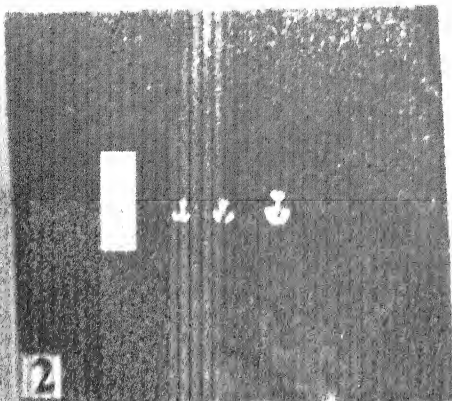
3



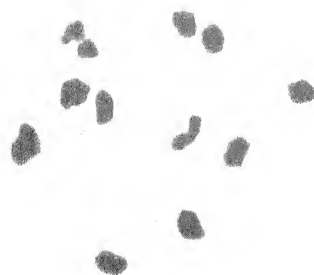
6

1

5



2



7

8



10 11 12 13 14 15 16 17 18 19 20

A. scarabaeoides x Caianus caian

Morphology.

Morphological observations on Atylosia scarabaeoides, Caianus caian and their hybrids (Table-94) are as follows:

1. Germination and first pair of leaves:

Both the parents, F_1 and F_2 's showed hypogeal germination. The shape of first pair of leaves was ovate in A. scarabaeoides and lanceolate in Caianus caian. The F_1 hybrid exhibited lanceolate shape of first pair of leaves.

Out of 10 F_2 plants studied, 8 showed lanceolate shape of first pair of leaves and remaining 2 had ovate shape.

2. Growth habit:

Atylosia scarabaeoides is a creeper and Caianus is an erect shrub. The cross between creeper and erect plant types resulted in F_1 hybrid showing intermediate growth habit (Plate-16; Fig. 5).

Out of 10 F_2 's selected for the present study, two were creeper, two erect and six had semierect growth habit.

3. Branching angle, stem and height:

Primary branches of Atylosia scarabaeoides and Caianus caian formed acute angle along their main stems. Likewise, F_1 hybrid also showed acute angled primary branches. At 50% flowering stage, A. scarabaeoides and C. caian possessed, on an average, 5 primary and 11 secondary branches, 5 primary and 17 secondary branches respectively; and the F_1 hybrid possessed 8 primary and 15 secondary

branches.

In both the parents as well as in the F_1 hybrid, the stem was green in colour with soft texture.

In the first year of growth A. scarabaeoides exhibited spread of 60 cm and C. cajan showed 103 cm height.

All the F_2 's exhibited acute angled primary branches. The number of primary branches ranged from 4 to 14 with 10.5 average primary branches and number of secondary branches ranged from 8 to 32 with 16.5 average secondary branches. In erect plants, stem height ranged from 95 to 122 cm and in creeping plants, spread ranged from 51 to 68 cm. In semi-erect plants, stem height ranged from 30 to 50 cm and spread ranged from 55 to 102 cm. Thus in F_2 's stem height ranged from 30 to 122 cm and spread of plant ranged from 51 to 102 cm, with 82.0 cm average stem height and 95.2 cm spread of plant in these F_2 's.

4. Leaf:

The leaflet shape in the case of A. scarabaeoides was obovate with acute leaf apices and in C. cajan, oval oblong with emarginate leaf apices. The F_1 hybrid showed intermediate shape of leaflet (Plate-14; Fig. 1). Seed parent and the F_1 showed hairy leaf surface while it was non hairy in C. cajan. In the F_1 hybrid, the average length and breadth of central leaflet was 3.7 cm and 1.5 cm respectively, whereas it were 2.5 and 1.4 cm in A. scarabaeoides and 4.6 and 2.0 cm in C. cajan. The average petiolar length in F_1 was found to be 1.7 cm while it was 1.6 cm in A. scarabaeoides and 2.6 cm in C. cajan.

In F_2 generation contrasting characters of leaf shape segregated. Two plants had obovate, 2 with oval oblong and 6 were shown to have intermediate leaf shape. In addition to trifoliate, unifoliate, bifoliate and quadrifoliate leaves were also observed (Plate-15; Fig. 20). Nine plants showed non-hairy leaf surface and one plant, hairy leaf surface. Leaf apices as acute, emarginate and intermediate types and leaf venation as palmately reticulate were seen in these plants. Central leaflet length and breadth ranged from 2.4 to 5.4 cm and 1.3 cm to 3.0 cm respectively. Petiolar length ranged from 1.5 to 3.1 cm with 2.21 cm average petiole length.

5. Days to flowering and maturity:

After sowing, flower bud initiation took place in 89, 90 and 98 days in A. scarabaeoides, C. cajan and their F_1 hybrid respectively. Days to 50% flowering were observed 100 in A. scarabaeoides, 105 in C. cajan and 123 in F_1 hybrid. On an average, the number of days taken from bud emergence to full development of flower were 9, 13 and 15 in A. scarabaeoides, C. cajan and F_1 hybrid respectively. The days taken between the pod initiation and maturity were 34, 37 and 39 days to 50% pod maturity were 142, 175 and 184 in A. scarabaeoides, C. cajan and F_1 hybrid respectively.

In F_2 's, duration of bud initiation ranged from 87 to 115 and the days from sowing to flowering ranged from 98 to 132. For full development of bud into flower, 9 to 15 days were taken and for pod initiation to pod maturity 35 to 43 days. Fifty per cent pod maturity period from the date of sowing, ranged from 150 to 185 days in F_2 's in the present study.

6. Flower:

The colour of the dorsal side of the standard petal was yellow with red stripes in A. scarabaeoides and yellow in C. cajan. The F_1 hybrid showed yellow with red strips standard petal colour (Plate-14; Fig. 2). Size of standard petal in F_1 was 1.08 cm^2 as against 0.401 cm^2 in A. scarabaeoides and 2.10 cm^2 in Cajanus (Table-94). The nature of the standard petal was persistent in A. scarabaeoides and deciduous in C. cajan, whereas, F_1 hybrid showed persistent nature of standard petals.

The colour of standard petal in 8 F_2 plants was yellow with red strips and 2 plants with yellow colour. Size of the standard petals ranged from 0.401 to 2.10 cm^2 with 1.56 cm^2 average size of standard petals. Nine plants comprised persistent and one, deciduous standard petal.

7. Pod setting:

The percentage of flowers to pods in F_1 hybrid was 10.0 as against 64.5 in A. scarabaeoides and 26.85 in Cajanus cajan (Table-94). In F_2 's, pod setting percentage ranged from 9.2 to 28.6 the average being 18.5%. Some of the F_2 's met with more pod setting percentage in comparison to F_1 hybrid.

8. Pod:

Colour of pod in A. scarabaeoides was green and in C. cajan green with black streaks. Pod colour in F_1 hybrid was uniformly brown. On an average, the pod size in seed parent pollen parent and their F_1 hybrid were 1.05, 3.78 and 1.75 cm^2 respectively. Pod shape was nearer to seed parent in F_1 hybrid (Plate-14; Fig. 3). Pods of A. scarabaeoides was hairy and that of Cajanus was non-hairy. Similar to seed

parent (A. scarabaeoides) F_1 hybrid showed hairy pods. Average pod thickness of F_1 hybrid was 0.40 cm as against 0.30 cm in A. scarabaeoides and 0.75 cm in C. caian.

In F_2 progeny, four plants had green and the remaining 6 possessed green associated with black streaks. The pod size ranged from 1.04 to 3.60 cm², the average being, 1.575 cm². Prominent and minute pod beaks were observed in 6 and 4 plants respectively. Eight plants with hairy pods and two plants with non-hairy pods were obtained. Thickness of pod ranged from 0.30 to 0.70 cm, the average being 0.355 cm. The nature of mature pods was shattering in A. scarabaeoides and non shattering in C. caian. In F_1 hybrid the mature pods showed shattering nature. In F_2 , 6 plants with non-shattering and four with shattering nature of mature pods were obtained.

9. Ovule fertility:

Percentage fertility of ovule was in the order of 20.0, 85.0 and 89.0 in F_1 hybrid, C. caian and A. scarabaeoides respectively. In F_2 , it ranged from 38.0 to 56.0 per cent with 41.5 per cent being average.

10. Seed:

The seed colour in female parent was brown with black dots and in male parent, brown. In F_1 hybrid, similar to female parent, brown with black dotted seeds were observed. Average seed thickness in A. scarabaeoides was 0.20 cm and in C. caianus 0.70 cm, while an intermediate seed thickness (0.302 cm) was recorded in F_1 hybrid. Chambers per pod on an average was found to be 3.2 in A. scarabaeoides, 2.9 in C. caian and 2.10 in F_1 hybrid. The average number of seeds per pod was 0.80 in the F_1 hybrid as against 2.5 in A. scarabaeoides and 2.2 in C. caian. Similar to seed parents F_1 hybrid possessed seed with prominent strophiole, whereas such character was altogether absent in C. caian.

In F_2 generation, 8 plants showed brown with black dots seed and 2 brown seed colour (Table-94). The seed thickness ranged from 0.20 to 0.50 cm, the average being 0.30 cm. Chambers per pod ranged from 2.0 to 5.00 with 2.86 average and seed per pod ranged from 0.9 to 3.2 with 1.41 seed average per pod. Strophioled seeds were obtained in 9 and non-strophioled seeds in one F_2 plant.

11. Stomata:

Stomatal sizes in A. scarabaeoides, C. cajan and F_1 hybrid was 108 μ , 189 μ and 180 μ respectively. Thus, stomatal size was intermediate in the F_1 hybrid (Plate-15; Fig. 12).

In F_2 's stomatal size ranged from 108 μ to 180 μ with a mean of 143 μ .

A. scarabaeoides x C. cajan F_1 hybrid.

Cytology

a) Mitosis:

The number of somatic chromosomes counted at metaphase was $2n = 22$ (Plate-14; Fig. 4). On the basis of total chromosome length, the somatic component of F_1 hybrid were grouped into 3 classes (Table-95). The classes A, B and C contributed by C. cajan and A_1 , B_1 and C_1 by Atylosia scarabaeoides. In the F_1 the somatic chromosomes from the two genomes were linearly arranged in pairs as per their length in descending order. The karyotypic description of each chromosome pair is as follows:

Chromosome pair 1:

Both the chromosomes differed from each other in short arm and total chromosome length by 0.02 μ and 0.02 μ

and 0.02 μ respectively. Their long arm length was similar but difference in position of primary constriction was observed. Secondary constriction was also observed on one of the chromosomes.

Chromosome pair 2:

The chromosome pair was similar in total length but difference was observed in short and long arm length by 0.03 μ and 0.03 μ respectively. These two chromosomes also differed with regard to position of primary constriction as one chromosome had median and the other had subterminal position of primary constriction.

Chromosome pair 3:

Both the chromosomes showed similar position of primary constriction but differ in short arm, long arm and total length by 0.28 μ , 0.25 μ and 0.03 μ respectively.

Chromosome pair 4:

Both the chromosomes differ in short arm, long arm and total length by 0.30 μ , 0.19 μ and 0.11 μ respectively. They also differ in position of primary constriction because one chromosome had median and other submedian primary constriction.

Chromosome pair 5:

Chromosomes of this pair did not differ with regard to position of primary constriction and total length of chromosome, but difference was observed in short arm and long arm length of 0.43 μ and 0.43 μ respectively.

Chromosome pair 6:

Both the chromosomes of this pair appeared to be homo-morphic with regard to position of primary constriction, short arm, long arm and total length of chromosomes.

Chromosome pair 7:

Identical chromosomes appeared to form this pair as they did not differ with regard to position of primary constriction, short arm, long arm and total chromosome length.

Chromosome pair 8:

These two chromosomes differed in short arm, long arm and total length by $0.61\ \mu$, $0.62\ \mu$ and $0.01\ \mu$ respectively. They also differed with regard to position of primary constriction as one chromosome possessed subterminal and other submedian primary constriction.

Chromosome pair 9:

The chromosomes of this pair did not differ with respect to position of primary constriction, short arm, long arm and total length of chromosomes and thus appeared to be identical.

Chromosome pair 10:

Difference was observed in short arm, long arm and total length of chromosomes by $0.43\ \mu$, $0.36\ \mu$ and $0.07\ \mu$ respectively. These two chromosomes also differed with regard to position of primary constriction (Table-95).

Chromosome pair 11:

Both the chromosomes differed in short arm, long arm and total length by 0.28 μ , 0.14 μ and 0.14 μ respectively. These two chromosomes also differed with regard to position of primary constriction as one chromosome possessed median and the other submedian primary constriction.

The total chromosome length ranged from 1.8 μ to 3.5 μ and the cumulative length of chromosome complement was observed to be 66.52 μ with 42.33% T.F.

Meiotic studies in F_1 hybrid of *Atylosia scarabaeoides* x *Cajanus cajan*.

Meiotic studies in F_1 hybrid revealed frequent formation of bivalents at diakinesis as well as at metaphase-I (Plate-14; Fig. 5). It can be seen from the table-96 that at metaphase-I ring bivalents ranged from 3-11 with 8.67 per cell and rod bivalents ranged from 0-8 with 2.05 per cell. Other than bivalents, quadrivalent, trivalent (Plate-14; Fig. 8) and univalents (Plate-14 Fig. 7) were also recorded. Quadrivalents ranged from 0-1 with 0.027 per cell and trivalents ranged from 0-2 with 0.092 per cell. Univalents ranged from 0-4 with 1.28 per cell at metaphase-I. Maximum number of four univalents were observed in 22.12 per cent of PMCs and two bivalents were observed in 3.68 per cent PMCs. One heteromorphic bivalent at metaphase-I was observed frequently (Plate-14; Fig. 6) at metaphase-I. Chiasma frequency as observed at diakinesis was 19.34 per cell and 1.93 per bivalent (Table-97).

At anaphase-I, 2 and 4 lagging chromosomes (Plate-15; Fig. 10) were observed in 1.60 and 1.66 per cent cells respectively. In remaining 91.30 per cent of cells normal

separation of chromosomes to the poles was observed. At the same stage single and double chromatid bridge (Plate-14; Fig. 9) was observed in 1.66 and 3.33 per cent of PMCs (Table-98).

At anaphase-II laggards were observed in 4.28% cells and in 95.14% cells equal separation was noticed (Table-99). At sporad stage, regular tetrad formation registered in 95.0% cells and in 5.0% of cells formation of micronuclei was observed (Table-99). Pollen fertility (Plate-15; Fig. 11) was 75.5%. Fertile pollen size ranged from 30-45 with 36.6 μ mean diameter.

Meiosis in F_2 plants.

Meiotic studies in 5 selected F_2 plants are as follows:

Plant No. 1:

It can be seen from the table-100 that at metaphase-I ring bivalents ranged from 3-11 with 8.67 per cell and rod bivalents ranged from 0-8 with 2.05 per cell. Bivalents were the only association in this plant. Chiasma frequency as observed at metaphase-I was 19.18 per cell and 1.82 per bivalent (Table-101). At anaphase-I and II (Table-102) equal separation was observed in all the PMCs studied. At sporad stage regular tetrad formation was observed. Pollen fertility percentage was 81.6. Fertile pollen size ranged from 30 to 45 μ with 39.0 mean diameter.

Plant No. 2:

At metaphase-I, bivalents and univalents were observed (Table-100). Ring bivalents ranged from 6-11 with 8.98 per cell and rod bivalents ranged from 0-4 with 0.95

per cell. Univalents ranged (Plate-15; Fig. 13) from 0-2 with 0.95 per cell. Chiasma frequency at metaphase-I was 19.44 per cell and 1.85 per bivalent (Table-101). At anaphase-I, one and three lagging chromosomes were observed in 3.07% and 3.07% of PMCs and in rest 93.33% normal separation of chromosomes was observed (Table-102). At anaphase-II laggards were observed in 4.0% cells while normal separation was observed in 96.0% cells (Table-103). At sporad stage formation of micronuclei (Plate-15; Fig. 17) was observed in 4.21% cells and in rest 95.55% cells regular tetrad formation was observed. Pollen fertility was 78.5% . Fertile pollen size ranged from 33 to 45 μ with 37.5 μ mean diameter.

Plant No. 3:

At metaphase-I ring and rod bivalents ranged from 7-11 and 0-4 with 9.3 and 0.82 per cell respectively (Table-100). Univalents (Plate-15; Fig. 15) ranged from 0-4 with 1.55 per cell. Chiasma frequency at metaphase-I was 18.92 per cell and 1.91 per bivalent (Table-101). At anaphase-I, two lagging chromosomes were observed in 5.71% cells and in 94.24% cells normal separation was observed in 94.24% cells (Table-102). At anaphase-II, laggards were observed in 6.66% of PMCs and in 93.24% PMCs normal separation was observed (Table-103). At sporad stage, formation of micronuclei was observed in 3.75% cells and in the rest 95.25% cells regular tetrad formation was observed. Pollen fertility percentage was 71.2 and fertile pollen size ranged from 33 to 45 μ with 39.6 μ mean diameter.

Plant No. 4:

Meiosis in this plant followed normal pattern as bivalents (Plate-15; Fig. 14) were the only chromosomal

association at metaphase-I (Table-100). Ring and rod bivalents ranged from 8-11 and 0-3 with 9.85 and 1.14 per cell respectively at metaphase-I. Chiasma frequency was 20.85 per cells and 1.89 per bivalent (Table-101). At anaphase-I and II regular separation was observed (Table-102) in all the cells studied. At sporad stage regular tetrad formation was observed. Pollen fertility was 88.5 and fertile pollen size ranged from 36 to 45 μ with 39.6 μ mean diameter.

Plant No. 5:

Normal meiosis was observed in this plant. At metaphase-I ring and rod bivalents (Plate-15; Fig. 16) ranged from 7-11 and 0-4 with 9.65 and 1.34 per cell respectively. Chiasma frequency at metaphase-I was 20.65 per cell and 1.87 per bivalent (Table-101). At anaphase-I and II normal disjunction of chromosomes was observed in all the PMCs studied. At sporad stage regular tetrad formation was observed. Pollen fertility (Plate-15; Fig.18) percentage was 88.5, fertile pollen size ranged from 36 to 45 μ with 39.6 μ mean diameter.

Table - 94

Morphological observations on Atylonia scarabaeoides, Cajanus cajan, their F_1 and F_2 hybrids.

| Characters | <u>A. scarabaeoides</u> | | <u>C. cajan</u> | | F_1 (One plant) | | F_2 's (10 plants) | |
|-----------------------------|-------------------------|--------------------|-----------------|----------------------|----------------------|---|----------------------|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Germination | | Hypogeal | Hypogeal | Hypogeal | Hypogeal | | | |
| Shape of first pair of leaf | | Ovate | Lanceolate | Lanceolate | Lanceolate | | | |
| Growth habit | | Herbaceous creeper | Erect shrub | Semi-erect spreading | Semi-erect spreading | | | |
| Branching | | Acute angled | Acute angled | Acute angled | Acute angled | | | |
| No. of primary branches | | 5 | 5 | 8 | 8 | | | |
| No. of secondary branches | | 11 | 17 | 15 | 15 | | | |
| Nature of stipules | | Persistent | Pugacious | Persistent | Persistent | | | |
| Central leaflet: shape | | Obovate | Oval oblong | Intermediate | Intermediate | | | |
| surface | | Hairy | Non-hairy | Hairy | Hairy | | | |
| length (cm) | | 2.5 | 4.6 | 3.7 | 3.7 | | | |
| breadth (cm) | | 1.4 | 2.0 | 1.5 | 1.5 | | | |
| venation | | palm. retic. | palm. retic. | palm. retic. | palm. retic. | | | |
| length of petiole (cm) | | 1.6 | 2.6 | 1.7 | 1.7 | | | |
| leaf apices | | Acute | Emerginate | Intermediate | Intermediate | | | |
| Stem: colour | | Green | Green | Green | Green | | | |
| woody/soft | | Soft | Soft | Soft | Soft | | | |

.....2.

| 1 | 2 | 3 | 4 | 5 |
|---|--|-----------------------------|---|--|
| Height/spread (cm) | 60 | 103 | Hg - 30 SP.-71 | Hg - 82.0 SP-95.2 100 118 14 34 |
| Days from sowing to bud initiation | 89 | 90 | 98 | |
| Days from sowing to flowering | 100 | 105 | 123 | |
| Days between bud to flower | 9 | 13 | 15 | |
| Days between bud initiation to maturity | 34 | 37 | 39 | |
| Flower: size of the standard petal (LxB) cm. colour of the standard petal | 0.8 x 0.51 yellow with red stripes | 1.5 x 1.4 yellow | 1.2 x 0.9 yellow with red stripes | 1.3 x 1.2 yellow with red stripes (8) yellow (2) persistent (9) deciduous (1) 1.3 |
| nature of petals | Persistent | Deciduous | Persistent | |
| length of style (cm) | 0.70 | 1.5 | 1.2 | |
| pod: colour of pod | Green | Green with black streaks | Brown | Green (4) Green with black streaks (6) 2.1 x 0.75 Present (8) Absent (2) Prominent (6) Minute (4) 0.355 Non-shatt. Shatt (4) |
| pod (L x B) cm hairs on mature pod | 2.1 x 0.50 Present | 5.4 x 0.7 Absent | 2.5x 0.70 Present | |
| beak of pod | Minute | Prominent | Inter- mediate | |
| thickness of pod nature of mature pod | 0.300 Shattering | 0.75 Non-shatt- ering | 0.400 Shattering | |

contd....3.

| | 1 | 2 | 3 | 4 | 5 |
|------------------------------|---|-----------------------|----------------|-----------------------|--|
| Seed: | | | | | |
| colour of seed | | Brown with black dots | Brown | Brown with black dots | Brown with black dots (8) Brown (2) |
| thickness of seed (cm) | | 0.200 | 0.702 | 0.302 | 0.300 |
| chambers per pod | | 3.2 | 2.9 | 2.10 | 2.86 |
| seed per pod | | 2.5 | 2.2 | 0.80 | 1.41 |
| strophiole | | Present | Absent | Present | Present (9) Absent (1) |
| Days to maturity | | 142 | 175 | 184 | 161 |
| Pod set (%) | | 64.5 | 26.85 | 10.0 | 18.5 |
| Ovule fertility (%) | | 89.0 | 85.0 | 20.0 | 41.50 |
| Stomata: | | | | | |
| Frequency (L x B) μ | | 6.8 12 x 9 | 7.0 18 x 12 | 7.0 15 x 12 | 8.0 12.6 x 11.4 |

(Figures in parentheses are No. of P_2 plants).

Table - 95

Observations on somatic chromosome complement of Atylosia
scarab x Cajanus cajan F₁ hybrid.

| S. No. | Class | Position of constriction | | Length of short arm (μ) | Length of long arm (μ) | Total chromo- some length (μ) | L/S arm ratio |
|-----------|----------------|-----------------------------|--------|-------------------------------------|------------------------------------|---|---------------------|
| | | Prim. | Secun. | | | | |
| 1 | A | M | | 1.77 | 1.77 | 3.54 | 1.00 |
| 2 | A ₁ | SM | SAT | 1.39+0.36 | 1.77 | 3.52 | 1.01 |
| 3 | A | M | | 1.75 | 1.75 | 3.50 | 1.00 |
| 4 | A ₁ | ST | | 0.72 | 2.78 | 3.50 | 3.86 |
| 5 | A | SM | | 1.70 | 1.80 | 3.50 | 1.05 |
| 6 | A ₁ | SM | | 1.42 | 2.05 | 3.47 | 1.44 |
| 7 | A | M | | 1.72 | 1.72 | 3.44 | 1.00 |
| 8 | A ₁ | SM | | 1.42 | 1.91 | 3.33 | 1.34 |
| 9 | A | SM | | 1.56 | 1.70 | 3.26 | 1.08 |
| 10 | A ₁ | SM | | 1.13 | 2.13 | 3.26 | 1.88 |
| 11 | A | SM | | 1.42 | 1.84 | 3.26 | 1.29 |
| 12 | A ₁ | SM | | 1.42 | 1.84 | 3.26 | 1.29 |
| 13 | A | SM | | 1.42 | 1.77 | 3.19 | 1.24 |
| 14 | A ₁ | SM | | 1.42 | 1.77 | 3.19 | 1.24 |
| 15 | A | ST | | 0.71 | 2.47 | 3.18 | 3.47 |
| 16 | A ₁ | SM | | 1.32 | 1.85 | 3.17 | 1.40 |
| 17 | B | SM | | 1.06 | 1.42 | 2.48 | 1.33 |
| 18 | B ₁ | SM | | 1.06 | 1.42 | 2.48 | 1.33 |
| 19 | B | M | | 1.06 | 1.06 | 2.12 | 1.00 |
| 20 | B ₁ | ST | | 0.63 | 1.42 | 2.05 | 2.25 |
| 21 | C | M | | 0.99 | 0.99 | 1.98 | 1.00 |
| 22 | C ₁ | SM | | 0.71 | 1.13 | 1.84 | 1.59 |

$$T.F. \% = \frac{28.16}{66.52} \times 100 = 42.33$$

Karyotypic Formula:

$$3A (M) + 11 A (SM) + 2A (ST) + 1B (M) + 2B (SM) + 1B (ST) + 1C (M) + 1C (SM)$$

Table - 96

Chromosome associations at Metaphase - I in Atylosia
scarabaeoides x Cajanus cajan (F₁ hybrid)

| No. of cells studied | Chromosome associations at Metaphase - I | | | | | Frequency | per cent |
|----------------------------|---|-----|------------|-----------|---|-----------|----------|
| | IV | III | Ring II | Rod II | I | | |
| 108 | 1 | - | 9 | - | - | 3 | 2.76 |
| - | - | 2 | 8 | - | - | 4 | 3.68 |
| - | - | 1 | 9 | - | 1 | 2 | 1.84 |
| - | - | - | 11 | 0 | - | 25 | 23.0 |
| - | - | - | 10 | 1 | - | 11 | 10.12 |
| - | - | - | 9 | 2 | - | 8 | 7.36 |
| - | - | - | 8 | 3 | - | 5 | 4.60 |
| - | - | - | 7 | 4 | - | 3 | 2.76 |
| - | - | - | 6 | 5 | - | 2 | 1.85 |
| - | - | - | 10 | - | 2 | 16 | 14.8 |
| - | - | - | 8 | 2 | 2 | 3 | 2.76 |
| - | - | - | 7 | 3 | 2 | 2 | 1.85 |
| - | - | - | 9 | 3 | 4 | 8 | 7.36 |
| - | - | - | 8 | 1 | 4 | 3 | 2.76 |
| - | - | - | 7 | 2 | 4 | 6 | 5.52 |
| - | - | - | 6 | 3 | 4 | 7 | 6.48 |

Range 0-1 0-2 6-11 0-5 0-4

Mean 0.027 0.092 9.07 0.89 1.27

Table - 97

Chiasma frequency in *Atylosia scarabaeoides* x *Cajanus cajan* (P_1 hybrid)

| Plant | Stage | No. of cells studied | No. of quadri-valents | No. of tri-valents | No. of bivalents with 2Xmata | No. of univalents | Total No. of chiasma-cells | Xmata per bivalent |
|--|------------|----------------------|-----------------------|--------------------|------------------------------|-------------------|----------------------------|--------------------|
| <u>A. scarab.</u> (♀ parent) | Diakinesis | 50 | - | - | 515 | 35 | 1065 | 21.3 1.93 |
| <u>C. cajan</u> (♂ parent) | Diakinesis | 50 | - | - | 508 | 42 | 1058 | 21.16 1.92 |
| <u>A. scarab.</u> x <u>C. cajan</u> (P_1 hybrid) | Diakinesis | 108 | 3 | 10 | 980 | 97 | 2089 | 19.34 1.93 |

Table - 98

Distribution of chromosomes at Anaphase - I in *Atylosia scarabaeoides* x *Cajanus cajan* (P_1 hybrid)

| Plant | No. of cells studied | Normal separation | No. of lagging chromosomes | | | | | Chromatid bridge | |
|--|----------------------|-------------------|----------------------------|-------------|---|-------------|---|------------------|-------------|
| | | | 1 | 2 | 3 | 4 | 5 | Single | Double |
| <u>A. scarab.</u> (♀ parent) | 70 | (100) | - | - | - | - | - | - | - |
| <u>C. cajan</u> (♂ parent) | 90 | (100) | - | - | - | - | - | - | - |
| <u>A. scarab.</u> x <u>C. cajan</u> (P_1 hybrid) | 60 | 55 (91.30) | - | 1 (1.60) | - | 1 (1.66) | - | 1 (1.60) | 2 (3.33) |

(Figures in parentheses are per cent)

Table - 99

Chromatid distribution at Anaphase - II in Atylosia scarabaeoides x Cajanus cajan
(F₁ hybrid).

| Plant | No. of cells studied | Normal separa- tion | Laggards | No. of cells studied | Tetrad | Micro- nuclei. | Pollen ferti- lity % | Fertile pollen size range (μ) | Mean (μ) |
|--|----------------------------|---------------------------|-------------|----------------------------|---------------|-------------------|-------------------------------|--|-------------------|
| <u>A. scarabaeoides</u> (♀ parent) | 80 | 80 (100) | - | 95 | 95 (100) | - | 99.4 | 30 - 33 | 31.5 |
| <u>C. cajan</u> (♂ parent) | 90 | 90 (100) | - | 85 | 85 (100) | - | 99.2 | 36 - 45 | 42.0 |
| <u>A. scarabaeoides</u> x <u>C. cajan</u> (F ₁ hybrid) | 70 | 67 (95.14) | 3 (4.28) | 120 | 114 (95.0) | 6 (5.00) | 75.5 | 30 - 45 | 36.6 |

(Figures in parentheses are per cent)

Table - 100

Chromosome associations at Metaphase - I in Atylosia
scarabaeoides x Cajanus cajan (F_2 plants)

| plant No. | No. of cells studied | Chromosome associations at M- I | | | Fre- quency | per cent |
|--------------|----------------------------|------------------------------------|-----------|------|----------------|----------|
| | | Ring II | Rod II | I | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1 | 79 | 11 | - | - | 32 | 40.50 |
| | | 10 | 1 | - | 16 | 20.25 |
| | | 9 | 2 | - | 5 | 6.3 |
| | | 8 | 3 | - | 7 | 8.82 |
| | | 7 | 4 | - | 8 | 10.12 |
| | | 6 | 5 | - | 6 | 9.52 |
| | | 4 | 7 | - | 3 | 3.78 |
| | | 3 | 8 | - | 2 | 2.53 |
| Range | | 3-11 | 0 - 8 | - | - | |
| Mean | | 8.67 | 2.05 | | | |
| 2 | 90 | 11 | - | - | 18 | 19.98 |
| | | 10 | 1 | - | 10 | 11.11 |
| | | 9 | 2 | - | 8 | 8.88 |
| | | 8 | 3 | - | 6 | 6.66 |
| | | 7 | 4 | - | 5 | 5.55 |
| | | 10 | - | 2 | 16 | 17.76 |
| | | 8 | 2 | 2 | 12 | 13.32 |
| | | 7 | 3 | 2 | 10 | 11.11 |
| | | 6 | 4 | 2 | 5 | 5.55 |
| Range | | 6-11 | 0-4 | 0-2 | | |
| Mean | | 8.98 | 1.46 | 0.95 | | |

contd....2.

- 2 -

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|-------|----|------|------|------|----|-------|
| 3. | 80 | 11 | 0 | - | 15 | 18.75 |
| | | 10 | 1 | - | 12 | 15.0 |
| | | 9 | 2 | - | 6 | 7.5 |
| | | 8 | 3 | - | 3 | 3.75 |
| | | 7 | 4 | - | 4 | 5.0 |
| | | 10 | - | 2 | 12 | 15.0 |
| | | 8 | 2 | 2 | 6 | 7.5 |
| | | 9 | - | 4 | 14 | 17.5 |
| | | 8 | 1 | 4 | 3 | 3.75 |
| | | 7 | 2 | 4 | 5 | 6.25 |
| Range | | 7-11 | 0-4 | 0-4 | | |
| Mean | | 9.3 | 0.82 | 1.55 | | |
| 4 | 61 | 11 | - | - | 21 | 34.23 |
| | | 10 | 1 | - | 16 | 26.2 |
| | | 9 | 2 | - | 18 | 29.50 |
| | | 8 | 3 | - | 6 | 9.83 |
| Range | | 8-11 | 0-3 | - | | |
| Mean | | 9.85 | 1.14 | | | |
| 5 | 83 | 11 | - | - | 30 | 36.0 |
| | | 10 | 1 | - | 20 | 24.0 |
| | | 9 | 2 | - | 15 | 18.0 |
| | | 8 | 3 | - | 10 | 12.0 |
| | | 7 | 4 | - | 8 | 9.6 |
| Range | | 7-11 | 0-4 | | | |
| Mean | | 9.65 | 1.34 | | | |

Table - 101

Chiasma frequency at Metaphase - I in Atylosia scarabaeoides x Calanus calan (F₂ plants)

| Plant No. | No. of cells studied | Bivalents with 2xmata | xmata with | No. of univalent | Total xmata | xmata per cell | xmata per bivalent |
|-----------|----------------------|-----------------------|------------|------------------|-------------|----------------|--------------------|
| 1 | 79 | 685 | 146 | - | 1516 | 19.18 | 1.82 |
| 2 | 90 | 809 | 132 | 86 | 1750 | 19.44 | 1.85 |
| 3 | 80 | 724 | 66 | 124 | 1514 | 18.92 | 1.91 |
| 4 | 61 | 601 | 70 | - | 1272 | 20.85 | 1.89 |
| 5 | 83 | 801 | 112 | - | 1714 | 20.65 | 1.87 |

10
11
12

Table - 102

Chromosome distribution at Anaphase - I in Atylosia scarabaeoides x Cajanus cajan
F₂ plants.

| Plant No. | No. of cells studied | Normal separa- tion | No. of lagging chromosomes | | | | | Chromatid bridge |
|--------------|----------------------------|---------------------------|----------------------------|-------------|-------------|---|-------------|---------------------|
| | | | 1 | 2 | 3 | 4 | 5 | |
| 1 | 62 | 62 (100) | - | - | - | - | - | - |
| 2 | 65 | 61 (93.33) | 2 (3.07) | - | 2 (3.07) | - | - | - |
| 3 | 70 | 66 (94.24) | - | 4 (5.71) | - | - | 3 (4.26) | - |
| 4 | 56 | 55 (98.21) | - | 1 (1.78) | - | - | - | - |
| 5 | 71 | 71 (100) | - | - | - | - | - | - |
| 6 | 80 | 80 (100) | - | - | - | - | - | - |

(figures in parentheses are per cent)

Table - 103

Chromatid separation at Anaphase - II in Atylosia scarabaeoides x Cajanus cajanF₂ plants.

| Plant No. | No. of cells studied | Normal separation | Laggs. | No. of cells studied | Quartet stage | | Pollen fertility % | Fertile pollen size | |
|-----------|----------------------|-------------------|------------|----------------------|---------------|---------------|--------------------|---------------------|----------|
| | | | | | Tetrad | Micro-nuclei. | | Range (μ) | Mean (μ) |
| 1 | 42 | 42 | - | 70 | 70 (100) | - | 84.6 | 30 - 45 | 39.0 |
| 2 | 50 | 48 (96.0) | 2 (4.0) | 95 | 91 (95.55) | 4 (4.21) | 78.5 | 33 - 45 | 37.5 |
| 3 | 45 | 42 | 3 | 80 | 77 (96.25) | 3 (3.75) | 71.2 | 33 - 45 | 39.6 |
| 4 | 51 | 51 (100) | - | 78 | 78 (100) | - | 85.7 | 36 - 45 | 38.4 |
| 5 | 55 | 55 (100) | - | 65 | 65 (100) | - | 88.5 | 36 - 45 | 39.6 |
| 6 | 60 | 60 (100) | - | 72 | 72 (100) | - | 91.2 | 30 - 45 | 42.3 |

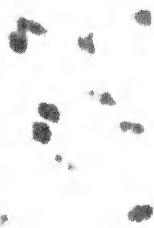
(figures in parentheses are per cent)

PLATE - 15 (A. scarabaeoides x C. cajan)

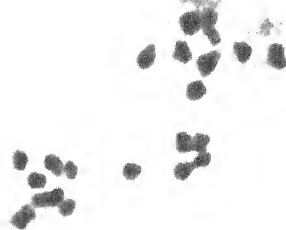
- Fig. 9. Double chromatid bridge at Anaphase-I of F_1 hybrid plant (X 1500)
- Fig. 10. Anaphase-I showing 5 laggaras in F_1 hybrid plant (X 1500)
- Fig. 11. Pollen grains of F_1 hybrid plant showing partial pollen sterility (X 600)
- Fig. 12. Stomata of F_1 hybrid plant showing variation in stomatal size (X 600)
- Fig. 13. 10 II's + 2 I's of F_2 hybrid Plant No. 2 (X 1500)
- Fig. 14. 11 bivalents of F_2 hybrid plant No. 4 at Metaphase-I (X 1500)
- Fig. 15. 10 II's + 2 I's F_2 plant No. 3, at Metaphase-I (X 1500)
- Fig. 16. 11 bivalents at Metaphase-I of F_2 plant No. 1 (X 1500)
- Fig. 17. Micronuclei with normal tetrads of F_2 plant No. 5 (X 600)
- Fig. 18. Pollen grains of F_2 plant No. 5 showing partial sterility (X 600)
- Fig. 19. Different types of leaves, flowers and pods of F_2 plants.
- Fig. 20. Single branch showing variation in leaf shape and leaflet number.

PLATE - 15

eraton)
Anaphase-1
wards to r_1 ho
plant sho
showing var
rid plant no.
plant no. 18
no. 3, at field
-3 of r_2 plant
erata of r_2 pl
no. 3 showing
flowers and
action in leaf



16

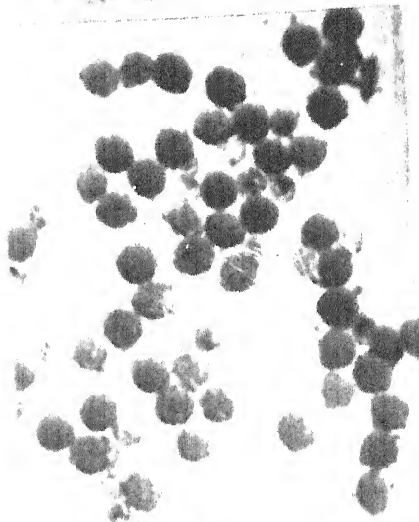


10

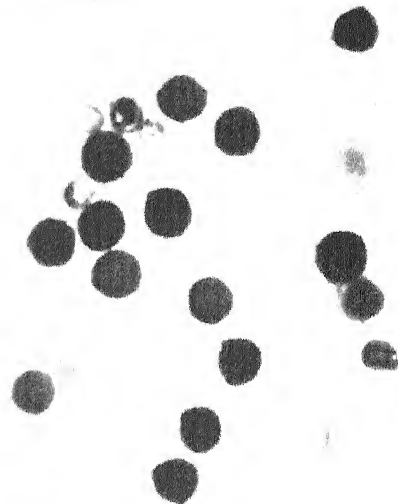
13



9



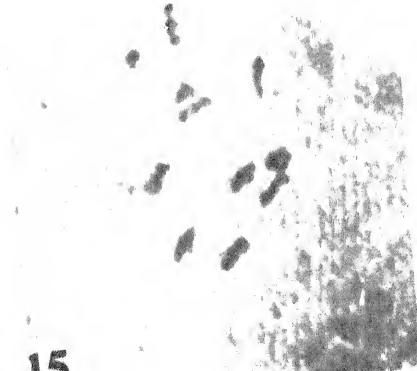
11



18



14



15



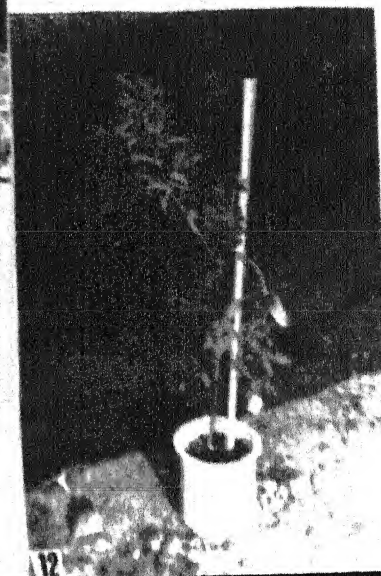
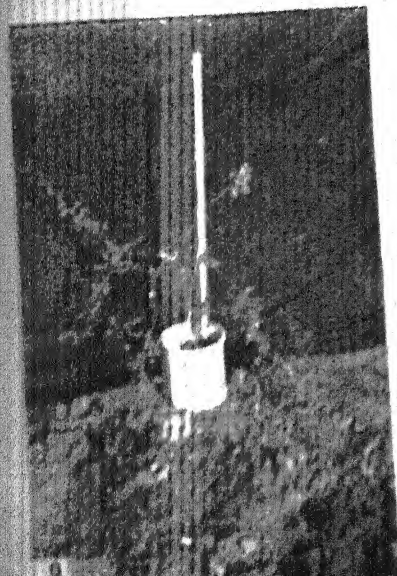
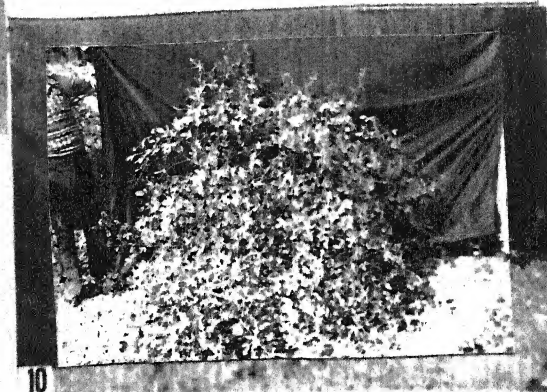
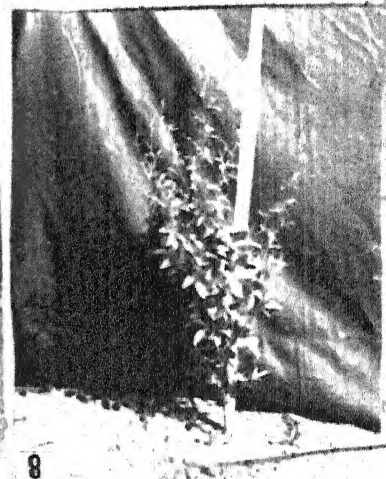
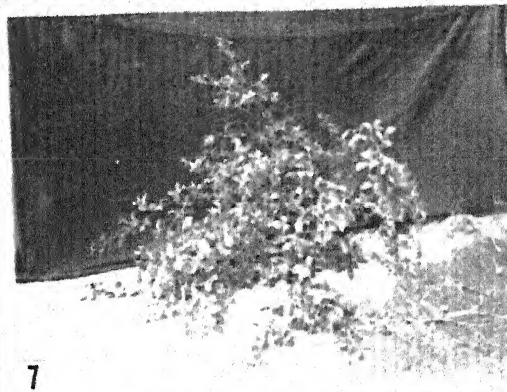
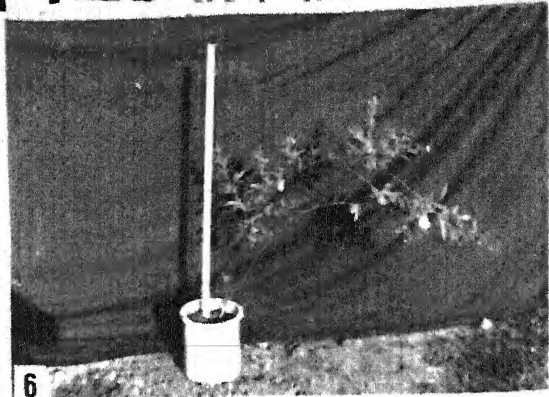
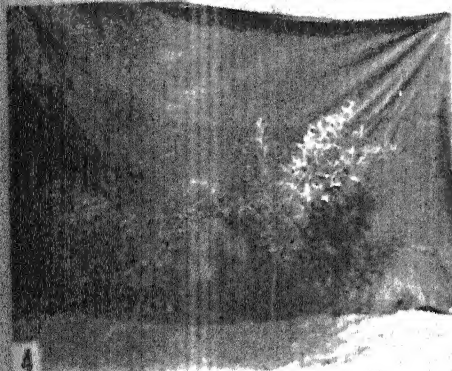
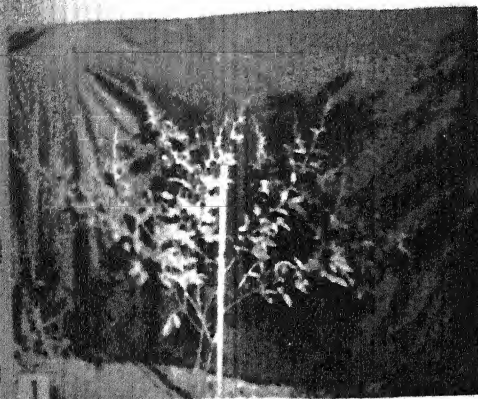
17



PLATE - 16

- Fig. 1. F_1 hybrid plant of A. lineata x C. cajan showing effect growth habit.
- Fig. 2. F_2 plant of A. lineata x Cajanus showing leaves from the base.
- Fig. 3. A tall F_2 plant of A. lineata x C. cajan.
- Fig. 4. One plant (L.) with nearly right angled branches and one plant (R) showing acute angled branches of A. lineata x C. cajan
- Fig. 5. F_1 hybrid plant of A. scarab. x C. cajan showing semierect growth habit.
- Fig. 6. F_2 plant of A. scarab. x C. cajan, showing erect but weak stem.
- Fig. 7. F_2 plant of A. scarab. x C. cajan showing semi-erect growth habit with spreading branches.
- Fig. 8. A dwarf erect F_2 hybrid plant of A. scarab. x C. cajan.
- Fig. 9. F_1 hybrid plant of A. albicans x C. cajan showing spreading growth habit.
- Fig. 10. F_2 hybrid plant of A. scarab. x C. cajan showing erect stem with some drooping branches.
- Fig. 11. F_2 plant of A. albicans x C. cajan showing erect stem from the base and spreading branches.
- Fig. 12. A F_2 plant of A. albicans x C. cajan showing one drooping branch.

PLATE - 16



INDUCTION OF POLYPLOIDY.

Observations on the effects of colchicine in *Atylosia platycarpa*.

a) Seed germination:

A summarised account on the effects of colchicine on seed germination at different concentrations and durations are presented in Table-104. The details are as follows:

The lowest concentration (0.05%) of colchicine used for 4, 6 and 8 hours showed no effects on seed germination. However, in the prolonged treatment for the period of 24 hours, a mild reduction in seed germination percentage was recorded (Table-104). 0.1% colchicine applied for 4, 6 and 8 hours showed no effect on seed germination but the same concentration of colchicine used for 24 hours exhibited only 20.0 per cent seed germination. When 0.2% colchicine solution applied for 2, 4, 6 and 8 hours, seed germination percentage was 90.0, 80.0, 40.0 and 10.0 respectively.

b) Plant survival:

The effects of colchicine on plant survival was studied after seed and seedling treatments (Table-104). Survival percentage differed in both the treatments. No plants could survive following seed treatment.

When seedlings were immersed in 0.05% aqueous colchicine solution for 4, 6 and 8 hours, percentage seedling survival was 16.0, 12.0 and 8.0 respectively (Table-104). Those seedlings immersed in 0.1% colchicine solution for 4, 6 and 8 hours when sown revealed 4.0 and 2.0 per cent survival respectively. When seedlings were immersed in 0.1% colchicine solution for 8 hours they could not survive. The

seedlings immersed in 0.2% aqueous colchicine solution for 2 hours, 4.0% seedling survival was noticed, those immersed in 0.2% solution for 4, 6 and 8 hours, could not survive.

Colchicine treatment of seedlings through absorbent cotton plug method exhibited differential survival of seedlings at different concentrations and durations. In the treatments of 0.05% colchicine for 8 hours a day, for one, two and three days, seedling survival percentage was 95.0, 90.0 and 80.0 respectively. 0.1 per cent solution, when applied for 8 hours a day for one, two and three days, the survival percentage of seedlings were 13.3, 5.88 and 2.94 respectively.

Production of polyploid:

Polyploid could not be induced in seed treatments. In seedling immersion treatment, one polyploid plant was obtained when seedlings were immersed in 0.1% colchicine solution for 6 hours. Chromosome doubling was also obtained in the apical bud treatment of seedlings through absorbent cotton plug soaked in 0.1 and 0.2% colchicine solutions used for 8 hours a day for, three days. In both the treatments with 0.1% and 0.2% colchicine solutions, percentage of tetraploid formed was 3.33 and 1.47 respectively.

Studied on induced tetraploids of *Alylosia platycarpa*.

A. Morphology:

Comparative morphological characters of diploid and induced tetraploids (C_0 and C_1) of *Alylosia platycarpa* are summarised in Table-105. Detail observations are as follows:

1. Seedling, branches and plant spread:

Immediately after treatment of the apical buds of the seedlings, the first pair of leaves gradually become darker green in colour and thicker than the untreated ones. The induced tetraploid of A. platycarnea showed poor vegetative growth with less number of primary as well as secondary branches. The average number of primary and secondary branches in diploid and tetraploid were 5 and 7; and 4 and 5 respectively. C_0 plant showed reduced plant spread (25.0)cm) as compared to diploid (32.5 cm). Stem of induced tetraploids had shorter internodes in comparison to its diploids.

In the C_1 generation, first pair of simple leaves were darker green in colour and thicker than their diploids. In C_1 plants the number of primary and secondary branches ranged from 6 to 14 and 9 to 16 respectively. Plant spread ranged from 33.0 cm to 39.0 cm, the average being 3.3 cm.

2. Days to flowering and maturity:

In contrast to diploids, delay in flowering as well as maturity was recorded in the induced tetraploids.

After sowing, C_0 plants took 60 days for bud initiation and 69 days to 50% flowering. Whereas, days for bud initiation and 50% flowering in diploid plants were 52 and 60 respectively. Mean number of days taken by buds for full development into flowers were 9.0 and 7.0 in tetraploid and diploid respectively. And the days between pod initiation and maturity were 30.0 and 27.0 in tetraploid and diploid respectively. Days to 50% pod maturity were observed to be 138 and 126 respectively.

In C_1 plants, days from sowing to bud initiation ranged from 52 to 57 and days from sowing to 50% flowering

60 to 70. The days taken by buds for full development into flowers ranged from 7.0 to 9.0 and days from pod initiation to maturity 27 to 32. Days to 50% pod maturity ranged from 128 to 133 in these C_1 plants.

3. Leaf:

The leaves of C_0 plants were comparatively thicker and darker green in colour. An increase in leaf size was observed in C_0 plants (Plate-17; Fig. 1) leaf length and breadth of tetraploids were 5.87 cm and 4.92 cm respectively and length and were 5.16 cm and 4.16 cm respectively breadth in diploids. The average petiolar length was 3.2 cm in tetraploids and 3.0 cm diploid. The surface of leaves of tetraploids was more hairy as compared to that of diploids.

In C_1 plants, leaf length and breadth ranged from 5.0 to 6.7 cm and 4.2 to 5.8 cm respectively. Petiolar length ranged from 3.0 to 3.5 cm with 3.3 cm being average, petiolar length. The leaves of C_1 plants were also thicker and darker green in colour. In all the C_1 plants, the surface of leaves was dense hairy.

4. Flower:

The C_0 plant produced larger flowers as compared to those of diploid. The size of standard petal of C_0 plant was 1.04 cm^2 as against 0.58 cm^2 in diploid (Plate-17; Fig. 2). Similarly the length of style was also increased as it was 1.2 cm in tetraploid and 1.0 cm in diploid.

In C_1 plants, the size of standard petals ranged from 1.02 to 1.21 cm^2 the average being 1.17 cm^2 . Stylor length ranged from 1.0 cm to 1.3 cm, the average being 1.1 cm.

5. Pod:

Tetraploids showed much reduced pod setting as compared to diploid. It was 5.0 per cent in tetraploid as against 74.0 in diploid. To categorise further, in C_1 plants, percentage pod setting ranged from 6.00 to 18.0, the average being 12.0 per cent.

Tetraploid plants had reduced pod size (Plate-17; Fig. 3) in comparison to diploid (3.51 cm^2 in tetraploids and 5.5 cm^2 in diploid). Pods of C_0 plants showed 0.400 cm average thickness while it was 0.308 cm in diploid. Pods of tetraploids were more hairy as compared to those of diploids. In tetraploids on an average number of chambers per pod and number of seeds per pod were 1.6 and 1.20 respectively. While in diploids, the number of chambers per pod and seeds per pod were 3.8 and 3.61 respectively.

In C_1 plants, pod sizes ranged from 3.2 to 4.0 cm^2 , the average being 3.70 cm^2 . Thickness of pods ranged from 0.36 to 0.48 cm the average being 0.415 cm. The number of chambers per pod ranged from 1 to 3 and number of seeds per pod 0.8 to 1.8, the average being 1.20 seeds per pod. All the C_1 plants possessed densely hairy pods.

6. Ovule fertility:

Observed percentage fertility of ovule was 66.0 in induced tetraploid (C_0) as against 93.6 in diploid. In C_1 plants, it ranged from 61.0 to 73.2%, the average being 68.0%.

7. Seed:

The seeds of C_0 plants were thicker and more bold in comparison to those of diploid. Average thickness. of

seeds of tetraploids was 0.35 cm as against 0.30 cm in diploid.

In C_1 plants, average seed thickness ranged from 0.35 to 0.48 cm, the average being 0.415 cm.

8. Stomata:

Considerable increase in the size of stomata (Plate-17; Figs. 4, 5) in tetraploid plants over the diploids was noticed. The length and breadth of stomata of C_0 plant was 21.0 μ and 15.0 μ respectively. While it was 12.0 μ and 9.0 μ in diploid, respectively. However, the tetraploid exhibited reduction in number of stomata per unit area (5.0) as compared to diploid (8.0).

In C_1 plants too, reduction in the number of stomata per unit area was observed and the mean value of 6.0 stomata per unit area was recorded. In these plants the size of stomata ranged from 270 μ to 318 μ , the average being 297 μ .

B. Cytology (C_0).

Mitosis:

Mitotic studies in root tip cells of colchicine treated seeds of A. platycarpa revealed different ploidy levels as 4n, 8n and 16n at different concentrations and durations (Table-106). The lowest concentrations (0.025%) of colchicine solution used for 6 hours brought about only condensation of chromosomes. While in 0.05% concentration and 6 hours duration of treatment 19.8% cells exhibited chromosome doubling (4n = 44) and in remaining cells (79.2%) 22 chromosomes were counted. Treatment with 0.1% colchicine solution for 6 hours duration resulted in the production of

60 and 30 per cent cells with $4n$ and $8n$ chromosome numbers respectively. The remaining 20.0 per cent cells exhibited $2n$ chromosome number. The treatment with 0.2% colchicine for 2 hours brought about 88.35%, 8.57% and 2.85%, $2n$; $4n$ and $8n$ cells, respectively. When 0.2% colchicine solution was applied for 4 hours, 80.0% and 20.0% cells with $4n$ and $8n$ chromosome were recorded respectively.

When 0.2% colchicine solution was used for 6 hours, the percentage of cells having more than $4n$ chromosomes viz, $8n$ and $16n$ were 52.0 and 5.26 respectively. Similarly, when the highest concentration of 0.2% colchicine solution applied for 8 hours, percentage of cells with $8n$ and $16n$ chromosomes (Plate-25; Figs. 11, 12, 13) were 75.9 and 17.8, respectively.

Meiosis:

Meiotic studies in C_0 plants revealed various chromosomal associations as quadrivalent, hexavalent (Plate-17; Figs. 6,9) pentavalent (Plate-17; Fig. 7) trivalent, bivalent and univalents at metaphase-I. It is clear from the table-107 that at metaphase-I hexavalent and pentavalent ranged from 0-1 and 0-1 with 0.085 and 0.057 per cell respectively. Quadrivalents and trivalents ranged from 0-11 and 0-1 with 5.33 and 0.057 per cell respectively. Bivalents and univalents ranged from 0-22 and 0-4 with 10.81 and 0.48 per cell respectively. 5.72% of PMCs showed formation of 11 quadrivalents (Plate-17; Fig. 8) and 5.2%, 22 bivalents. Maximum number 4 univalents were recorded in 2.86% cells. Chiasma frequency at metaphase-I was 40.30 per cell (Table-9). At anaphase-I, laggards were observed in 2.32% cells and in remaining 97.67% cells, equals separation of chromosomes to the poles was observed (Table-110). At sporad stage, regular tetrad

formation was observed in 96.25% cells, except in 3.75% cells where micronuclei were formed.

Pollen fertility was 72.13% and fertile pollen size ranged from 36.0 μ to 48 μ with 42.0 μ mean diameter. While in diploids pollen size ranged from 30-36 μ . (Plate-17; Figs. 11, 12).

Cytology (C₁).

a) Mitosis:

Mitotic study revealed 44 somatic chromosomes (Plate-17; Fig. 14).

b) Meiosis:

Meiotic studies were carried out in 3 selected tetraploid plants (Table-108) and the observations are as follows:

Plant No. 1:

Studies on chromosomal associations at M-1 revealed pollen grain mother cells with varying number of quadrivalents and bivalents (Table-108). In this plant, quadrivalents (Plate-17; Fig. 13) ranged from 0-6 with 2.6 per cell, maximum number of 6 IVs were noticed in 30.0% cells. At metaphase-I, bivalents and univalents ranged from 10-22 and 0-6 with 16.3 and 1.00 per cell respectively. Maximum number of 22 bivalents and 6 univalents were observed in 30.0% and 10.0% cells respectively. Chiasma frequency as observed at metaphase-I, was 41.57 per cell. At anaphase-I equal separation of chromosomes to the poles was observed in all the cells studied (Table-110). At sporad stage regular tetrad formation was observed.

Pollen fertility was 92.5% and fertile pollen size ranged from 39 to 48 μ with 40.7 μ mean diameter.

Plant No. 2:

At metaphase-I multivalents and bivalents were noticed (Table-108). The quadrivalents ranged from 3-8 with 6.41 per cell and bivalents 6-16 with 9.17 per cell. Maximum number of 8 quadrivalents and 16 bivalents were recorded in 26.70 and 7.14% cells respectively. Chiasma frequency at metaphase-I was 41.80 per cell (Table-109). At anaphase-I, regular disjunction of chromosomes to the poles and thereafter regular formation of tetrads were noticed (Table-110).

Pollen fertility was 96.7% and fertile pollen size ranged from 42 to 51 μ with 42.2 μ mean pollen diameter.

Plant No. 3:

In this plant, quadrivalents, bivalents and univalents were recorded at metaphase-I. The quadrivalents ranged from 2-8 with 5.71 per cell. Maximum number of 8 quadrivalents registered in 24.48 per cent cells. Bivalents and univalents ranged from 6-18 and 0-2 with 10.36 and 0.40 per cell respectively. Maximum number of 18 bivalents were recorded in 8.16% cells (Table-108). Chiasma frequency as observed at metaphase-I was 41.04 per cell. At anaphase-I, lagards were seen in 3.63% of cells and in remaining cells equal separation of chromosomes to the poles was observed (Table-110). At sporad stage formation of tetrad was observed in 94.32% cells and in 5.26% micronuclei were observed.

Pollen fertility percentage was 93.4 and fertile pollen size ranged from 36 to 48 μ with 41.8 mean diameter.

Table - 104

Effects of colchicine on seed germination and plant survival in Alysicarpus platycarpus. (% in parenthesis). No. of seeds treated in each case were = 10.

| Seed treatment | | | Seedling treatment (Immersion method) | | | | Seedling treatment (through absorbent cotton plug method) | | | | | |
|---------------------------|-------------------------|--------------------------|---------------------------------------|-------------------------|-------------------------------------|--------------------------------|---|---------------------------|---------------------------------|--|---------------------------------|--------------------------|
| Concen- tration (%) | Dura- tion (hrs.) | Seeds germi- nated | Concen- tration (%) | Dura- tion (hrs.) | No. of seed- lings treated | Seed- ling survi- ved | Tetra- ploid | Concen- tration (%) | Dura- tion (hrs/ days) | No. of seed- lings treat- ed | Seed- lings survi- ved | Tetra- ploid plant |
| 0.05 | 4 | 10 (100) | 0.05 | 4 | 25 | 4 (16.0) | - | 0.05 | A ₁ | 20 | 19 (95.0) | - |
| 0.05 | 6 | 10 (100) | 0.05 | 6 | 25 | 3 (12.0) | - | 0.05 | A ₂ | 20 | 18 (90.0) | - |
| 0.05 | 8 | 10 (100) | 0.05 | 8 | 25 | 2 (8.0) | - | 0.05 | A ₃ | 20 | 16 (80.0) | - |
| 0.05 | 24 | 9 (90.0) | 0.1 | 4 | 50 | 2 (4.0) | - | 0.1 | A ₁ | 30 | 18 (60.0) | - |
| 0.1 | 4 | 10 (100) | 0.1 | 6 | 50 | 1 (2.0) | 1 (2.0) | 0.1 | A ₂ | 30 | 16 (53.3) | - |
| 0.1 | 6 | 10 (100) | 0.1 | 8 | 50 | 0 | - | 0.1 | A ₃ | 30 | 8 (26.6) | 1 (3.33) |
| 0.1 | 24 | 2 (20.0) | 0.2 | 2 | 25 | 1 (4.0) | - | 0.2 | A ₁ | 45 | 6 (13.3) | - |
| 0.2 | 2 | 9 (90.0) | 0.2 | 4 | 25 | 0 | - | 0.2 | A ₂ | 51 | 3 (5.88) | - |
| 0.2 | 4 | 8 (80.0) | 0.2 | 6 | 25 | 0 | - | 0.2 | A ₃ | 68 | 2 (2.94) | 1 (1.47) |
| 0.2 | 6 | 4 (40.0) | | | | | | | | | | |
| 0.2 | 8 | 1 (10.0) | | | | | | | | | | |

A₁ = 8 hrs - One day; A₂ = 8 hours - 2 days; A₃ = 8 hours - 3 days.

Table - 105

Comparative morphological observations in diploid and induced tetraploid of Atylosia platycarpa.

| Characters | <u>A. platycarpa</u> | <u>A. platycarpa</u> | <u>A. platycarpa</u> |
|---|----------------------|----------------------|-----------------------|
| | 2x | 4x (C ₀) | 4 x (C ₁) |
| 1 | 2 | 3 | 4 |
| No. of primary branches | 5 | 4 | 8 |
| No. of secondary branches | 7 | 5 | 12 |
| Central leaflet: | | | |
| surface | Hairy + | Hairy ++ | Hairy ++ |
| (L x B) cm. | 5.16 x 4.16 | 5.87 x 4.92 | 6.0 x 5.0 |
| length of petiole (cm.) | 3.0 | 3.2 | 3.3 |
| plant spread (cm.) | 32.5 | 25.0 | 35.0 |
| Days from sowing to bud initiation | 52 | 60 | 54 |
| Days from sowing to flowering | 60 | 69 | 65 |
| Days between bud to flower | 7 | 9 | 8 |
| Days between pod initiation to maturity | 27 | 30 | 29 |
| Size of the standard petal (L x B) cm. | 0.97 x 0.60 | 1.30 x 0.80 | 1.31 x 0.90 |

contd....2.

| 1 | 2 | 3 | 4 |
|--|-------------------|--------------------|--------------------|
| Length of style (cm.) | 1.0 | 1.2 | 1.2 |
| Pod (L x B) cm. | 5.0 x 1.1 | 3.51 x 1.0 | 3.7 x 1.0 |
| Thickness of pod (cm.) | 0.308 | 0.400 | 0.415 |
| Hairs on mature pod | Present + | Present ++ | Present ++ |
| No. of chambers per pod | 3.8 | 1.6 | 1.8 |
| No. of seeds per pod | 3.61 | 1.20 | 1.30 |
| Thickness of seed (cm.) | 0.30 | 0.350 | 0.360 |
| Days to maturity | 126 | 138 | 130 |
| Pod set (%) | 74.0 | 5.0 | 12.0 |
| Ovule fertility (%) | 93.6 | 66.6 | 68.0 |
| Stomata: frequency (L x B) μ | 8.0 12.0 x 9.0 | 5.0 21.0 x 15.0 | 6.0 19.8 x 15.0 |

Table - 106

Effect of colchicine on somatic chromosomes of Axylosia platycarpa (C₀)
 (% values in parentheses)

| concentration (%) | duration (hours) | No. of cells studied | ploidy level at metaphase | | | |
|----------------------|---------------------|-------------------------|---------------------------|---------------|--------------|-------------|
| | | | 2n | 4n | 8n | 16n |
| 0.025 | 6 | 25 | 25 (100) | - | - | - |
| 0.05 | 5 | 30 | 24 (79.2) | 6 (19.8) | - | - |
| 0.1 | 6 | 40 | 8 (20.0) | 20 (50.0) | 12 (30.0) | - |
| 0.2 | 2 | 35 | 31 (88.35) | 3 (8.57) | 1 (2.85) | - |
| " | 4 | 25 | - | 20 (80.0) | 5 (20.0) | - |
| " | 6 | 38 | - | 16 (42.10) | 20 (52.0) | 2 (5.26) |
| " | 8 | 28 | - | 2 (6.66) | 23 (75.9) | 5 (17.8) |

22
23
23

Table - 107

Chromosome associations at Metaphase - I in induced
tetraploid of Atylosia platycarpa (C₀)

| Chromosome associations at M-I | | | | | | frequency Per cent | |
|--------------------------------|---|----|-----|----|---|--------------------|-------|
| VI | V | IV | III | II | I | | |
| 1 | - | 8 | - | 3 | - | 3 | 4.29 |
| 1 | - | 7 | - | 5 | - | 3 | 4.29 |
| - | 1 | 6 | 1 | 5 | 2 | 2 | 2.86 |
| - | 1 | 5 | - | 9 | 1 | 2 | 2.86 |
| - | - | 11 | - | - | - | 4 | 5.72 |
| - | - | 10 | - | 2 | - | 11 | 15.73 |
| - | - | 9 | - | 4 | - | 8 | 11.44 |
| - | - | 6 | - | 10 | - | 7 | 10.01 |
| - | - | 3 | - | 16 | - | 3 | 4.29 |
| - | - | 2 | - | 18 | - | 5 | 7.15 |
| - | - | 1 | - | 20 | - | 5 | 7.15 |
| - | - | 1 | - | 19 | 2 | 5 | 7.15 |
| - | - | 1 | - | 18 | 4 | 2 | 2.86 |
| - | - | - | 1 | 20 | 1 | 2 | 2.86 |
| - | - | - | - | 22 | - | 4 | 5.72 |
| - | - | - | - | 21 | 2 | 4 | 4.72 |

Range 0-1 0-1 0-11 0-1 0-22 0-4

Mean 0.085 0.057 5.33 0.057 10.81 0.48

Table - 108

Chromosome associations in induced tetraploids of Atylosia platycarpa (C_1)

| Plant No. | No. of cells studied | Chromosome associations at M - I | | | | Frequency | Per cent |
|-----------|----------------------|----------------------------------|-----|-------|------|-----------|----------|
| | | IV | III | II | I | | |
| 1 | 40 | 6 | - | 10 | - | 12 | 30.0 |
| | | 3 | - | 15 | 2 | 8 | 20.0 |
| | | 2 | - | 18 | - | 4 | 10.0 |
| | | - | - | 22 | - | 12 | 30.0 |
| | | - | - | 19 | 6 | 4 | 10.0 |
| Range | | 0-6 | - | 10-22 | 0-6 | | |
| Mean | | 2.6 | - | 16.3 | 1.00 | | |
| 2 | 56 | 8 | - | 6 | - | 15 | 26.70 |
| | | 7 | - | 8 | - | 12 | 21.42 |
| | | 6 | - | 10 | - | 18 | 32.14 |
| | | 5 | - | 12 | - | 7 | 12.46 |
| | | 3 | - | 16 | - | 4 | 7.14 |
| Range | | 3-8 | - | 6-16 | - | | |
| Mean | | 6.41 | - | 9.17 | - | | |
| 3 | 49 | 8 | - | 6 | - | 12 | 24.48 |
| | | 7 | - | 8 | - | 8 | 16.32 |
| | | 6 | - | 10 | - | 9 | 18.36 |
| | | 6 | - | 9 | 2 | 7 | 14.28 |
| | | 3 | - | 16 | - | 6 | 12.24 |
| | | 2 | - | 18 | - | 4 | 8.16 |
| | | 2 | - | 17 | 2 | 3 | 6.12 |
| Range | | 2-8 | - | 6-18 | 0-2 | | |
| Mean | | 5.71 | - | 10.36 | 0.40 | | |

Table - 109

Chiasma frequency at metaphase - I in induced tetraploids of Atylosia platycarpa

| Genera- tion | No. of cells studied | No. of VI | No. of V | No. of Quadrivalents with 3xmata 4xmata | No. of triva- lents | No. of Bivalents with 2xmata 1xma lents | No. of total xmata univa- xmata per lents cell |
|----------------------------------|----------------------------|--------------|-------------|--|---------------------------|--|--|
| C ₀ | 70 | 6 | 4 | 50 | 324 | 4 | 607 150 34 2821 40.30 |
| C ₁ Plant No. 1 | 40 | - | - | 5 | 99 | - | 600 52 40 1663 41.57 |
| Plant No. 2 | 56 | - | - | 9 | 350 | - | 400 114 - 2341 41.80 |
| Plant No. 3 | 49 | - | - | 20 | 260 | - | 403 105 20 2011 41.04 |

Table - 110

Chromosome distribution at Anaphase - I in induced tetraploid of *Atylonia platycarpa*

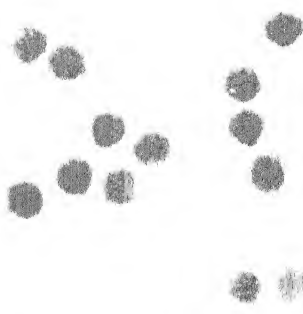
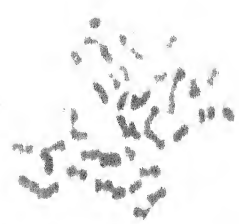
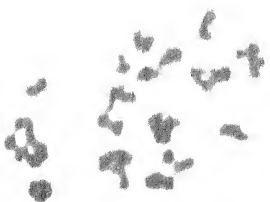
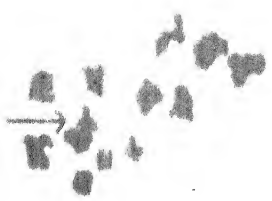
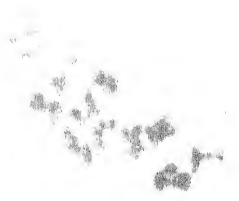
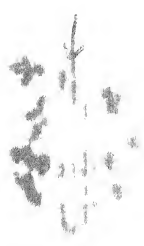
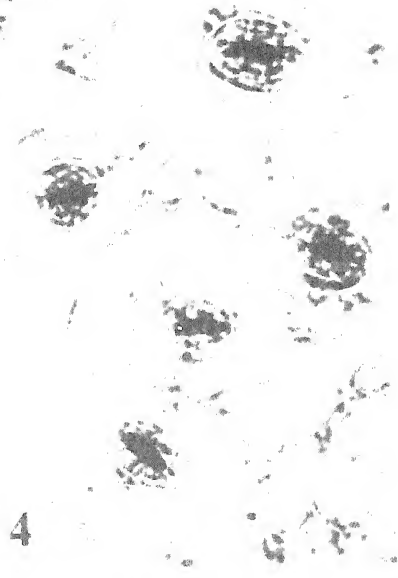
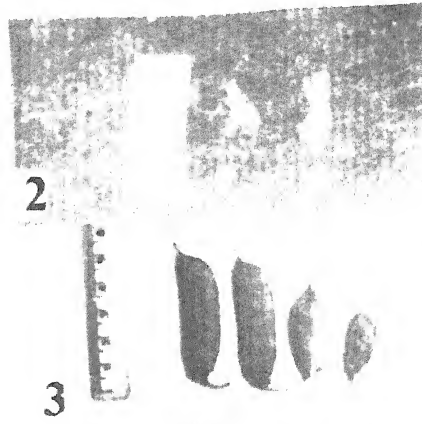
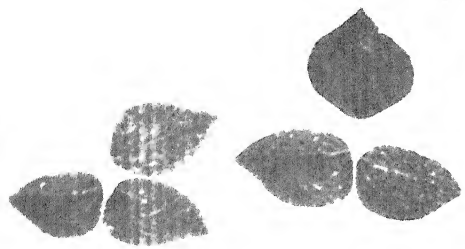
| Genera- tion | No. of cells studied | Anaphase - I | | No. of cells studied | Sporad Stage | | Pollen ferti- lity % | Fertile pollen size | |
|----------------------------------|----------------------------|--------------------------|----------------------------|----------------------------|---------------|----------------------------|-------------------------------|------------------------|-------------------|
| | | equal separa- tion | unequal separa- tion | | Tetrad | Pol- Micro- yad nuclei. | | Range (μ) | Mean (μ) |
| C ₀ | 43 | 42 (97.67) | - | 1 (2.32) | 77 (96.25) | - 3 (3.75) | 72.13 | 36-48 | 42.0 |
| C ₁ Plant No. 1 | 65 | 65 (100) | - | - | 85 (100) | - | 92.5 | 39-48 | 40.7 |
| Plant No. 2 | 60 | 60 (100) | - | - | 92 (100) | - | 96.7 | 42-51 | 43.2 |
| Plant No. 3 | 55 | 53 (96.36) | - | 2 (3.63) | 72 (94.32) | - 4 (5.26) | 93.4 | 36-48 | 41.8 |

(Figures in parentheses are per cent)

PLATE - 17 (Induced tetraploid of A. platycarpa)

- Fig. 1. Leaves of diploid and tetraploid (Left to right)
- Fig. 2. Flowers of diploid and tetraploid (Left to Right)
- Fig. 3. Pods of diploid and tetraploid (Left to Right)
(2 in each case)
- Fig. 4. Stomata of diploid (X 600)
- Fig. 5. Stomata of tetraploid (X 600)
- Fig. 6. 1 VI + 8 II's + 3 II's at Metaphase-I (X 1500)
- Fig. 7. 1 V + 6 IV's + 1 KKK + 5 II's + 2 I's at
Metaphase-I (C_0) (X1500)
- Fig. 8. 11 IV's at Metaphase-I (C_0) (X 1500)
- Fig. 9. 1 IV + 7 IV's + 5 II's at Metaphase (C_0) (X 1500)
- Fig. 10. Pentad with normal telzads at sporad stage (C_1)
(X 400)
- Fig. 11. Pollen grains of diploid (40 X 15)
- Fig. 12. Pollen grains of tetraploids (40 X 15).
- Fig. 13. 6 IV's + 10 II's at Metaphase-I of C_1 plant
No. 1 (X 1500)
- Fig. 14. 44 somatic chromosomes at Metaphase-I of C_1
Seed (X 1500)

PLATE - 17



Observations on the effects of colchicine on *Atylosia albicans*.

a) Seed germination:

The effects of colchicine on seed germination at different concentrations and durations of treatments are as follows:

In the treatment with 0.05% colchicine for 4, 6 and 8 hours, germination of all the seeds was observed (Table-111). When the treatment prolonged to 24 hours, only 30.0% seed germination was recorded. Application of 0.1% colchicine for 4, 6, 8 and 24 hours, revealed 100%, 90.0%, 80.0% and 20.0% seed germination respectively. In the treatment of 0.2% colchicine for 2, 4, 6 and 8 hours, seed germination was observed to be 90.0%, 90.0%, 80.0% and 60.0% respectively.

b) Plant survival:

The effects of colchicine on plant survival was studied in seed and seedling treatments (Table-111). Survival percentage differed in both the treatments. The survival percentage of plants recorded after seed treatment varied from 0-20%. The highest survival percentage (20%) having been recorded in treatment of 0.05% colchicine applied for 4 hours. In the treatment of 0.05% colchicine for 6 hours 10.0% plant survival was observed. In the treatment with 0.05% colchicine solution for 8 and 24 hours, plants could not survive. Whereas, in the treatment of 0.1% colchicine for 4 hours 10.0% plant survival was recorded, and in longer duration treatments viz., 6, 8 and 24 hours, plants could not survive. Similarly in the treatments with 0.2% colchicine for 2, 4 6 and 8 hours durations, plants could not survive. The seed treatment was not successful. In the treated seeds, seedling could not develop properly after the respective

treatments. The plants which survived after the respective treatments were noticed to be diploid on their cytological examination.

When seedlings were immersed in 0.05% aqueous colchicine solution for 4, 6 and 8 hours, survival percentage of seedlings were 90.0%, 60.0% and 25.0% respectively. Seedlings immersed in 0.1% colchicine solution for period of 4, 6 and 8 hours, 44.0%, 16.0% and 8.0% were observed to survive respectively. 0.2% colchicine solution when used for 2, 4 and 6 hours, showed 12.0%, 8.0% and 2.0% seedling survival respectively. Those seedlings immersed in 0.2% colchicine solution for 8 hours, could not recover from the toxic effects of the alkaloid, hence no plant could be obtained.

Colchicine treatment of seedlings through absorbent cotton plug method exhibited differential survival of seedlings at different concentrations and durations. All the seedlings survived after the treatment with 0.5% colchicine for 8 hours a day for one, two and three days. In the treatment with 0.1% for 8 hours a day for one, two and three days, percentage survival was 100.0, 83.33 and 90.0 respectively.

c) Production of polyploid:

Chromosome doubling was successfully induced through the apical bud treatment wherein seedlings were immersed in 0.2% colchicine solution, for 6 hours duration and as well as when the apical buds were treated through the absorbent cotton plug soaked in 0.2% aqueous colchicine solution for 8 hours a day for 3 days.

210

Studies on induced tetraploids of *Atylosia albicans*.

a) Morphology:

Comparative morphological characters of diploid and induced tetraploids of *Atylosia albicans* (C_0 and C_1) are summarised in table-112. Details on morphological observations pertaining to diploid and induced tetraploids of *A. albicans* are as follows:

1. Seedling, branches and plant spread:

After treatment of the apical buds of the seedlings, the first pair of leaves gradually become darker green in colour and thicker than the untreated ones. The induced tetraploid of *A. albicans* in C_0 generation, showed less number of primary and secondary branches in comparison to its diploid counterpart. The number of primary and secondary branches in diploid and tetraploid plants were 10 and 14; 3 and 5 respectively.

In the C_1 generation, first pair of simple leaves was darker green in colour and thicker than their diploids. In C_1 plants, the number of primary and secondary branches ranged from 8 to 18 and 14 to 26 respectively. In the C_1 plants, spread ranged from 90 cm to 105 cm with 95.0 cm average plant spread.

2. Days to flowering and maturity:

Delayed flowering and maturity were observed in tetraploids in contrast to diploids.

After sowing, the C_0 plant took 177 days for bud initiation and 190 days for 50% flowering. Whereas, the diploid plants took, on an average, 152 and 170 days

respectively for bud initiation and 50% flowering. Average number of days taken ^{by} buds for full development into flower in diploid and C_0 plant were 12 and 15 respectively and the durations between pod initiation to pod maturity were 36 and 40 days respectively.

In C_1 plants, on an average, days from sowing to bud initiation ranged from 165 to 175 and days from sowing to flowering ranged from 187 to 199. The average number of days taken by bud for full development into flower ranged from 12.0 to 16.0 and the days between pod initiation to pod maturity ranged from 35.0 to 42.0 and in these C_1 plants days to 50% pod maturity ranged from 268 to 281.

3. Leaf:

The leaves of C_0 plants were thicker and darker green in colour in contrast to the diploid. Marked increase in length and breadth of leaves in C_0 plant was noticed in comparison to its diploid (Plate-18; Fig. 1). The central leaf let length and breadth in C_0 plant was 6.3 cm and 4.7 cm respectively as against 4.1 cm length and 3.0 cm breadth in diploid. Similarly, increased petiolar length was observed (3.8 cm in C_0 plant and 3.5 cm in diploid plant). On the surface of leaves visible hairs were absent in the diploid as well as C_0 plant.

In C_1 generation, central leaf let length and breadth of tetraploid plants ranged from 6.0 cm to 7.4 cm and 4.0 cm to 5.6 cm respectively. Petiolar length in these plants ranged from 3.0 to 4.8 cm, the average being 3.9 cm petiolar length. Comparatively the leaves of C_1 plants were also thicker and darker green in colour. In all the C_1 plants the leaves were non-hairy.

4. Flower:

The C_0 plant produced larger flowers as compared to diploids. The size of standard petal of C_0 plant was 3.06 cm^2 as against 2.56 cm^2 of the diploid. Similarly, on an average, the length of style was found to be increased in the tetraploid over the diploid (Table-112).

In C_1 plants, the size of the standard petal ranged from 3.02 to 3.44 cm^2 , the average being 3.24 cm^2 . In these plants an increase in stylon length was also observed in comparison to diploid (Table-112).

5. Pod:

The induced tetraploid (C_0) of Atylosia albicans showed 5.76% pod setting as against 62.0% in diploid. In C_1 plant it ranged from 8.6% to 28.5%, the average being 17.5%.

Tetraploid plant had reduced pod size in comparison to diploid as it was 2.0 cm^2 in tetraploid (C_0) and 1.76 cm^2 in the diploid. Number of chambers per pod was observed to be 2.6 and 2.0 in diploid and tetraploid respectively. In C_1 plant marked reduction in number of seeds per pod was noticed (Table-112). Thickness of pod was 0.40 cm in tetraploid and 0.35 cm in diploid. Pods of diploid as well as tetraploid were non-hairy.

In C_1 plants, pod size ranged from 1.8 to 2.3 cm^2 , the average being 2.20 cm^2 . Pod thickness ranged from 0.37 cm to 0.46 cm, the average being 0.42 cm^2 . In these plants, number of chambers per pod ranged from 1-4 and the number of seeds per pod ranged from 0.7 to 1.8, the average being 1.0. Pods of C_1 plants were observed to be non-hairy.

7. Seeds:

The ovule fertility percentage was 29.16 in induced tetraploid (C_0) and 73.0% in the diploid plant. In C_1 plants it ranged from 31.2% to 56.0%, the average being 43.0%.

The seeds of C_0 plant were held in comparison to the diploid. Average thickness of seeds was 0.36 cm in C_0 plants and 0.28 cm in the diploid. In C_1 plants, seed thickness ranged from 0.300 cm to 0.400 cm, the average being 0.37 cm.

8. Stomata:

An increase in the size of stomata of tetraploid over the diploid was noticed. The length and breadth of stomata of C_0 plant was 15.0 μ and 12.0 μ respectively. Whereas, it was 12.0 μ and 9.0 μ in diploid plant. However, the tetraploid exhibited reduction in number of stomata per unit area (6.0) as compared to diploid (8.0).

In C_1 plants too, reduction in the number of stomata per unit area was observed and the mean value of 6.2 stomata per unit area was recorded. In these C_1 plants, the size of stomata ranged from 154 μ to 192 μ with 171.0 μ being the average (Table-112).

b) Cytology (C_0).

Mitosis:

Mitotic studies in root tips cells of colchicine treated seeds of A. albicans revealed different ploidy levels (4n and 8n) (Plate-24; Figs. 1,2,3) at different concentration and durations (Table-113). The minimum concentration of 0.025% colchicine solution used for 6 hours

brought about only condensation of chromosomes. While at 0.05% concentration and 6 hours duration of treatment, 19.8% cells showed chromosome doubling ($2n = 4x = 44$), and the remaining 79.92% cells showed $2n = 22$. In the treatment with 0.1% concentration for 6 hours duration the diploid chromosome number 22 and $4x$ chromosome number (44) were recorded in 28.57% and 71.42% of somatic cells respectively. When 0.2% colchicine solution was applied for 2 hours, 44 chromosomes were observed in 8.0% cells and in remaining 92.0% cells 22 chromosomes were observed. In the other treatment with the same strength of the chemical used for 4 hours, 22 and 44 chromosomes were observed in 86.8% and 13.2% cells respectively, and for 6 hours, $4n$ and $8n$ ploidy levels were observed in 92.0% and 8.0% cells respectively. The highest concentration of 0.2% colchicine applied for 8 hours resulted in more than 44 chromosomes. In the remaining 75.0% cells 44 chromosomes were observed. All the somatic cells exhibited polyploidy in the treatment of 0.2% colchicine for 6 and 8 hours duration (Table-113).

Meiosis:

Meiotic study in C_0 plant revealed various chromosomal associations at $M-I$, viz., quadrivalent, bivalent and univalents. It is clear from the Table-114, that at metaphase-I, quadrivalents ranged from 0-8 with 6.0 per cell. At this stage of meiosis maximum number of 8 IVs were observed in 27.7% of cells. Maximum percentage of cells (30.47) were observed with chromosomal association of 6 IVs + 10 IIs (Plate-18; Fig. 3). At metaphase-I, bivalents ranged from 6-20 with 9.91 per cell and univalents ranged from 0-4 with 0.16 per cell. Maximum number of 4 univalents were observed in 2.77% cells. Chiasma frequency as observed at metaphase-I was 41.76 per cell (Table-116). At anaphase-I, unequal distribution of chromosomes to the poles was observed in 4.70% cells and in remaining 95.23%

cells, equal separation to the poles was observed (Table-117). At sporad stage, tetrads and micronuclei (Plate-18; Fig.5) were observed in 92.43% and 7.03% of cells respectively.

Pollen fertility percentage was 61.2 and fertile pollen size (Plate-18; Figs.6,7) ranged from $42\ \mu$ to $48\ \mu$ with $45.4\ \mu$ mean diameter. In diploids, fertile pollen size ranged from $33\text{--}39\ \mu$.

Cytology (C_1).

a) Mitosis:

Mitotic study of C_1 plants revealed $2n = 4x = 44$ (Plate-18; Fig. 11) at somatic metaphase of root tip cells.

b) Meiosis:

Meiotic studies were carried out in two selected tetraploid plants and the observations are presented here.

Plant No. 1:

Observations on chromosomal associations at metaphase-I revealed PMCs with varying number of quadrivalents, bivalents and univalents at metaphase-I (Table-115). In this plant quadrivalents ranged from 0-6 with 3.42 per cell. Maximum number of 6 quadrivalents were recorded in 34.78% of cells. An association of 5 IVs + 12 IIs was observed in 17.39% cells. At metaphase-I bivalents and univalents ranged from 0-22 and 0-44 with 13.6 and 2.0 per cell respectively. Maximum number of 22 bivalents (Plate-18; Fig. 4) were observed in 21.70% cells and maximum number of 44 univalents were observed in 4.34% cells at metaphase-I. Chiasma frequency was 37.47 per cell (Table-116). At anaphase-I, equal separation of chromosomes to the poles

Table - 111

Effects of Colchicine on seed germination and plant survival in Arylonia albicans (JM 2337).
(% in parentheses) No. of seeds treated in each case were 10.

| Seed treatment | | | Seedling treatment (Immersion method) | | | Seedling treatment (drop through cotton plug method) | | | tetraploid plants | | |
|---------------------------|-------------------------|---|---------------------------------------|-------------------------|--|--|---------------------------|---------------------------|--------------------------------|--|--------------------------------|
| Concen- tration (%) | Dura- tion (hrs.) | Seed germi- nation survi- ved | Concen- tration (%) | Dura- tion (hrs.) | No. of seed- lings treat- ed | Seed- lings survi- ved | Tetra- ploid plants | Concen- tration (%) | Dura- tion hrs./ days | No. of seed- lings treat- ed | Seed- ling survi- ved |
| 0.05 | 4 | 10 (100) | 0.05 | 4 | 20 | 18 (90.0) | - | 0.05 | A ₁ | 20 | 20 (100) |
| 0.05 | 6 | 10 (100) | 0.05 | 6 | 20 | 12 (60.0) | - | 0.05 | A ₂ | 20 | 20 (100) |
| 0.05 | 8 | 10 (100) | 0.05 | 8 | 20 | 5 (25.0) | - | 0.05 | A ₃ | 20 | 20 (100) |
| 0.05 | 24 | 3 (30.0) | - | - | - | - | - | - | - | - | - |
| 0.1 | 4 | 10 (100) | 0.1 | 4 | 50 | 22 (44.0) | - | 0.1 | A ₁ | 30 | 30 (100) |
| 0.1 | 6 | 9 (90.0) | 0.1 | 6 | 50 | 8 (16.0) | - | 0.1 | A ₂ | 30 | 28 (93.33) |
| 0.1 | 8 | 8 (80.0) | 0.1 | 2 | 50 | 4 (8.0) | - | 0.1 | A ₃ | 30 | 27 (90.0) |
| 0.1 | 24 | 2 (20.0) | 0.2 | 2 | 50 | 6 (12.0) | - | 0.2 | A ₁ | 55 | 46 (83.63) |
| 0.2 | 2 | 9 (90.0) | 0.2 | 4 | 50 | 4 (8.0) | - | 0.2 | A ₂ | 70 | 51 (72.85) |
| 0.2 | 4 | 9 (90.0) | 0.2 | 6 | 50 | 1 (2.0) | 1 | 0.2 | A ₃ | 35 | 9 (25.7) |
| 0.2 | 6 | 8 (80.0) | 0.2 | 8 | 50 | 0 | (2.0) | - | - | - | (2.0) |
| 0.2 | 8 | 6 (60.0) | - | - | - | - | - | - | - | - | - |

(% in parentheses)

A₁ = 8 hrs. - One day; A₂ = 8 hrs. 2 days; A₃ = 8 hrs. 3 days.

Table - 112
Comparative morphological observations in diploid and tetraploid *Atylosia albicans*.

| Characters | <u>A. albicans</u> | | <u>A. albicans</u> | |
|--|--------------------|----------------------|----------------------|--|
| | 2x | 4x (C ₀) | 4x (C ₁) | |
| No. of primary branches | 10 | 3 | 12 | |
| No. of secondary branches | 14 | 5 | 16 | |
| Central leaflet: surface | Non-hairy | Non-hairy | Non-hairy | |
| (L x B) cm. | 4.1 x 3.0 | 6.3 x 4.7 | 6.5 x 4.8 | |
| length of petiole (cm.) | 3.50 | 3.8 | 3.90 | |
| spread of plant (cm.) | 92.0 | 30.0 | 95.0 | |
| Days from sowing to bud initiation | 152 | 177 | 170 | |
| Days from sowing to flowering | 170 | 190 | 192 | |
| Days between bud to flower | 12 | 15 | 13 | |
| Days between pod initiation to maturity | 36 | 40 | 38 | |
| Size of the standard petal (L x B) cm. | 1.6 x 1.6 | 1.8 x 1.7 | 1.8 x 1.8 | |
| length of style (cm.) | 1.6 | 1.7 | 1.8 | |
| Pod (L x B) cm. | 2.2 x 0.8 | 2.0 x 1.0 | 2.2 x 1.0 | |
| Thickness of pod (cm.) | 0.35 | 0.40 | 0.42 | |
| Hairs on mature pod | Absent | Absent | Absent | |
| No. of chambers per pod | 2.60 | 2.0 | 2.3 | |
| No. of seeds per pod | 2.20 | 0.58 | 1.0 | |
| Thickness of seed (cm.) | 0.28 | 0.36 | 0.37 | |
| Days to maturity | 268 | 281 | 275 | |
| Pod set (%) | 62.0 | 5.76 | 17.50 | |
| Ovule fertility (%) | 73.0 | 29.16 | 43.0 | |
| Stomata, frequency (L x B) / μ | 9.0 | 6.0 | 6.2 | |
| | 12.0 x 9.0 | 15.0 x 12.0 | 14.8 x 11.6 | |

Table - 113

Effects of Colchicine on somatic chromosomes of Atylosia albicans (% in parentheses)

| Concentration (%) | Duration (Hours) | No. of cells studied | PLOIDY LEVEL AT METAPHASE | | | |
|-------------------|------------------|----------------------|---------------------------|--------------|--------------|-----|
| | | | 2n | 4n | 8n | 16n |
| 0.025 | 6 | 34 | 34 (100) | - | - | - |
| 0.05 | 6 | 31 | 24 (80.92) | 6 (18.8) | - | - |
| 0.1 | 6 | 28 | 20 (71.4) | 8 (28.57) | - | - |
| 0.2 | 2 | 25 | 23 (92.0) | 2 (8.0) | - | - |
| " | 4 | 30 | 26 (86.8) | 4 (13.2) | - | - |
| " | 6 | 25 | - | 23 (92.0) | 2 (8.0) | - |
| " | 8 | 40 | - | 30 (75.0) | 10 (25.0) | - |

11100

Table - 114

Chromosome associations at Metaphase - I in induced tetraploid of Atylosia albicans (C₀)

| No. of cells studied | Chromosome associations at M - I | | | | Frequency | Per cent |
|----------------------|----------------------------------|-----|------|------|-----------|----------|
| | IV | III | II | I | | |
| 72 | 8 | - | 6 | - | 20 | 27.7 |
| | 7 | - | 8 | - | 6 | 8.31 |
| | 7 | - | 7 | 2 | 2 | 2.77 |
| | 6 | - | 10 | - | 22 | 30.47 |
| | 5 | - | 12 | - | 8 | 11.11 |
| | 4 | - | 14 | - | 8 | 11.11 |
| | 3 | - | 16 | - | 4 | 5.55 |
| | - | - | 20 | 4 | 2 | 2.77 |
| Range | 0 - 8 | - | 6-20 | 0-4 | | |
| Mean | 6.0 | - | 9.91 | 0.16 | | |

Table - 115
Chromosome associations at Metaphase - I in induced
tetraploid of Atylosia albicans (C₁)

| Plant no. | No. of cells studied | Chromosomal associations at M - I | | | | Frequ- ency | Per cent |
|--------------|----------------------------|--------------------------------------|-----|------|------|----------------|----------|
| | | IV | III | II | I | | |
| 1 | 46 | 6 | - | 10 | - | 16 | 34.78 |
| | | 5 | - | 12 | - | 8 | 17.39 |
| | | 3 | - | 16 | - | 6 | 13.02 |
| | | - | - | 22 | - | 10 | 21.70 |
| | | - | - | 20 | 2 | 2 | 4.34 |
| | | - | - | - | 44 | 2 | 4.34 |
| Range | | 0-6 | - | 0-22 | 0-44 | | |
| Mean | | 3.41 | | 13.6 | 2.0 | | |
| 2 | 53 | 8 | - | 6 | - | 12 | 22.64 |
| | | 7 | - | 7 | 2 | 8 | 15.04 |
| | | 6 | - | 10 | - | 14 | 26.32 |
| | | 6 | - | 9 | 2 | 3 | 5.66 |
| | | 6 | - | 8 | 4 | 4 | 7.52 |
| | | 5 | - | 12 | - | 5 | 9.40 |
| | | 4 | - | 14 | - | 4 | 7.52 |
| | | 2 | - | 18 | - | 3 | 5.66 |
| Range | | 2-8 | - | 6-18 | 0-4 | | |
| Mean | | 6.13 | - | 9.52 | 0.41 | | |

Table - 116

Chiasma frequency at M - I in induced tetraploid of Alylosia albicans

| Generation | No. of cells studied | Quadrivalents with 3xmata | 4xmata | No. of trivalents | No. of bivalents with 2xmata | 1xma | No. of univalent | Total xmata per cell |
|----------------------------|----------------------|---------------------------|--------|-------------------|------------------------------|------|------------------|----------------------|
| C ₀ | 72 | 35 | 397 | - | 600 | 124 | 12 | 3007 41.76 |
| C ₁ Plant No. 1 | 46 | 4 | 150 | - | 500 | 112 | 92 | 1724 37.47 |
| Plant No. 2 | 53 | 25 | 300 | - | 400 | 105 | 22 | 2180 41.13 |

Table - 117

Chromosome distribution at Anaphase - I in induced tetraploid of Alylosia albicans.

| Generation | No. of cells studied | Normal separation | Unequal distribution | Laggards | No. of cells studied | Sporad stage of tetrad | Micro-nuclei | Pollen fertility % | Fertile pollen size Range (μ) Mean (μ) |
|----------------------------|----------------------|-------------------|----------------------|----------|----------------------|------------------------|--------------|--------------------|--|
| C ₀ | 42 | 40 (95.23) | 2 (4.76) | - | 85 | 79 (92.43) | 6 (7.05) | 61.2 | 42 - 48 45.4 |
| C ₁ Plant No. 1 | 45 | 45 (100) | - | - | 90 | 85 (94.35) | 5 (5.55) | 73.2 | 42 - 48 45.1 |
| Plant No. 2 | 40 | 37 (92.5) | 3 (7.5) | - | 76 | 74 (97.36) | 2 (2.60) | 81.6 | 42 - 48 46.3 |

(figures in parentheses are per cent)

PLATE - 18 (Induced tetraploid of A. albicans)

Fig. 1 to 7 of C_0 plant.

Fig. 1. Leaves of diploid and tetraploid (Left to Right)

Fig. 2. 7 IV's + 7 II's + 2 I's at Metaphase-I (C_0)

Fig. 3. 6 IV's + 10 II's at Metaphase-I (C_0)

Fig. 4. 22 II's at Metaphase-I (No. 1) C_1 (X 1500)

Fig. 5. Micronuclei at sporad stage (C_0) (X 400)

Fig. 6. Pollen grains of diploid (X 400)

Fig. 7. Pollen grains of tetraploid (X 400)

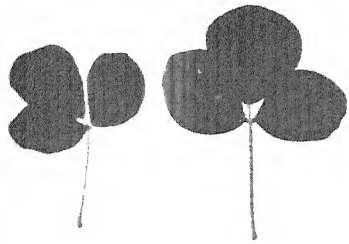
Fig. 8. 7 IV's + 8 II's at Metaphase-I (C_1) No. 2. (X 1500)

Fig. 9. 3 IV's + 14 II's + 4 I's (C_1) No. 2 (X 1500)

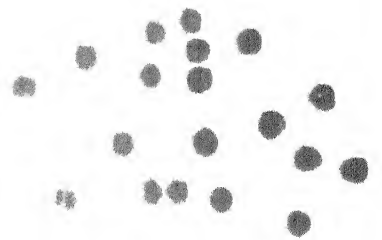
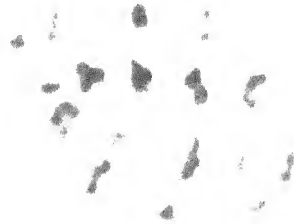
Fig. 10. 22-22 at each pole of anaphase-I No. 1 (C_1) (X 1500)

Fig. 11. 44 somatic chromosomes (C_1) (X 1500)

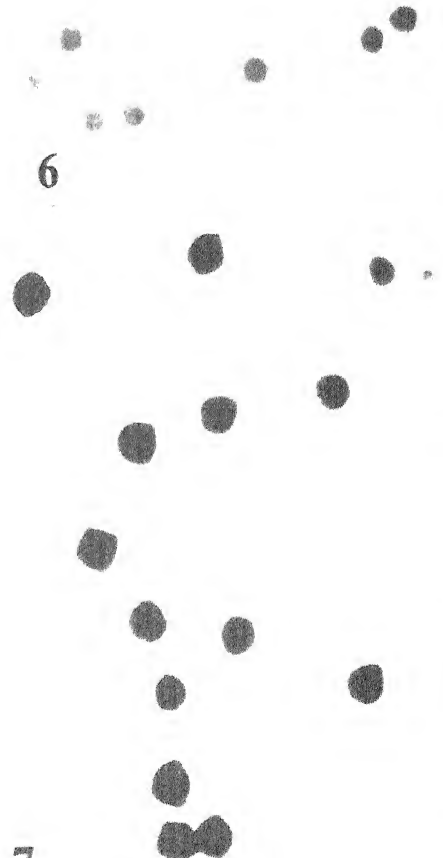
PLATE - 18



3



6



7

4



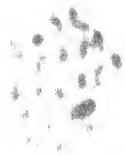
2



8

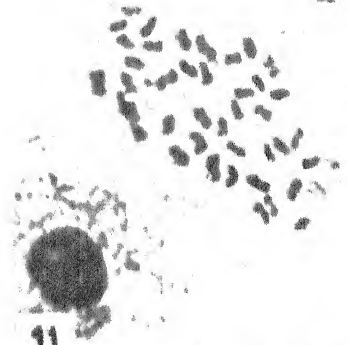


9



5

11



was observed in all the cells studied (Plate-18; Fig. 10). At sporad stage tetrads and micronuclei were observed in 94.35% and 5.55% cells respectively (Table-117).

Pollen fertility was 73.2% and the fertile pollen size ranged from 42 μ to 48 μ with 45.1 μ mean diameter.

Plant No. 2:

In this plant chromosomal association at metaphase-I exhibited varying number of quadrivalents, bivalents and univalents (Plate-18; Fig. 9) (Table-115). The quadrivalents ranged from 2-8 with 6.13 per cell at metaphase-I. Bivalents and univalents ranged from 6-18 and 0-4 with 9.52 and 0.41 per cell respectively. Maximum number of 8 IVs were observed in 22.64% of cells, and 7 quadrivalents were observed in 15.04% cells (Plate-18; Fig. 8). Chiasma frequency as observed at metaphase-I was 41.13 per cell (Table-116). At anaphase-I unequal distribution of chromosomes to the poles was noticed in 7.5% cells and in remaining 92.5% cells, equal separation of chromosomes to the poles was observed (Table-117). At sporad stage tetrads and micronuclei were formed in 97.36% and 2.60% cells respectively. Pollen fertility percentage was 81.6 and the fertile pollen size ranged from 42 to 48 μ with 46.3 μ mean diameter.

Observations on the effects of colchicine on *Atylosia lineata*.

a) Seed germination:

The effects of colchicine on seed germination at different concentrations and durations are as follows:

In the treatment with 0.05% colchicine for 4, 6 and 8 hours durations, germination of all the seeds was

observed (Table-118). When the treatment prolonged to 24 hours, only 10% seed germination was observed. The treatments with 0.1% colchicine for 4, 6 and 8 hours, showed no effect on seed germination. But in the prolonged treatment of 24 hours, inhibiting effect on seed germination was noticed as only 60.0% seeds could germinate. In the treatment of 0.2% colchicine for 2, 4, 6 and 8 hours, seed germination was 90.0, 90.0, 80.0 and 70.0 per cent, respectively. The time taken by treated seeds for germination varied from 2-6 days while the untreated seeds germinated in 2-4 days.

b) Plant survival:

The effects of colchicine on plant survival was studied in seeds and seedling treatments (Table-118). Survival percentage differed in both of the treatments. In seed treatment, plant survival varied from 0-20%. The highest survival percentage (20.0) having been recorded with 0.05% colchicine when applied for 6 hours. In the treatment with 0.05% for 4 hours, 10% plants survived and when 0.05% colchicine applied for 8 and 24 hours, plants could not survive. In the treatment with 0.1% colchicine for 4 hours, survival of 10.0% plants was recorded. When 0.1% colchicine applied for 6, 8 and 24 hours, plants could not survive after treatments. Similarly in the treatments with 0.2% colchicine for 2, 4, 6 and 8 hours duration plants could not survive. The seed treatment was not successful, the chief cause of failure appeared to be the drastic effect of colchicine on roots, which later on failed to produce lateral roots and hence seedlings could not develop properly after the respective treatments. The plants which survived after the treatments were found to be diploid on their cytological examination. When seedlings were immersed in 0.05% aqueous colchicine solution for 4, 6 and 8 hours,

percentage seedling survival observed was 60.0, 40.0 and 20.0 per cent, respectively. Seedlings treated with 0.1% colchicine solution for periods of 4, 6 and 8 hours, 25.0, 20.0 and 10.0 per cent seedlings survived respectively. When seedlings were immersed in 0.2% colchicine solution for 2, 4, and 6 hours, percentage survival observed was 25.0, 10.0 and 3.33 per cent respectively. Those seedlings immersed in 0.2% colchicine solution for 8 hours could not recover from the toxic effects of the alkaloid, thus, no plants could be obtained.

Colchicine treatment of seedlings through absorbant cotton plug method exhibited differential survival of seedlings at different concentrations and durations. When 0.05% colchicine applied for 8 hours a day for one, two and three days, all the seedlings survived. In the treatment with 0.1% colchicine for 8 hours a day for one, two and three days, percentage survival was 95.0, 90.0 and 85.0 respectively. When 0.2% colchicine applied for 8 hours a day for one, two and three days, 90.0, 55.0 and 4.0 per cent seedlings survived.

6) Production of polyploid:

In the seed treatment with 0.05% and 0.1% colchicine for periods of 4, 6, 8 and 24 hours and 0.2% for 2, 4, 6 and 8 hours polyploid plant could not be obtained. Similarly in the immersion method, when seedlings were treated with 0.05%, 0.1% and 0.2% colchicine for 4, 6 and 8 hours tetraploid plants could not be obtained. However, chromosome doubling was successfully induced through the apical bud treatment wherein colchicine solution of 0.2% strength was applied for 8 hours a day for 2 days.

Studies on induced tetraploids of *Atylosia lineata*.

a) Morphology:

Comparative morphological characters of diploid and induced tetraploids of *Atylosia lineata* (C_0 and C_1) are summarised in Table-119. Detail observations pertaining to the morphology of diploid and induced tetraploids of *A. lineata* are as follows:

1. Seedling, branches and stem height:

Immediately after treatment of the apical buds of the seedlings, the first pair of leaves gradually become darker green in colour and thicker than the untreated ones. The induced tetraploid of *A. lineata* showed less number of primary and secondary branches in comparison to its diploid counterpart. The number of primary and secondary branches in diploid and tetraploid plants were 5 and 8, 2 and 3 respectively. C_0 tetraploid plant showed reduced height (56.0 cm) as compared to diploid (110 cm). Stem of induced tetraploid was thicker with shorter internodes in comparison to its diploid.

In the C_1 generation, first pair of simple leaves were darker green in colour and thicker than their diploids. In C_1 plants, the number of primary and secondary branches ranged from 3 to 12 and 5 to 16 respectively and stem height ranged from 105 to 121 cm with 116 cm average plant height.

2. Days to flowering and maturity:

Delayed flowering and consequent maturity were observed in tetraploids in contrast to diploids.

After sowing, the C_0 plant taken 127 days for bud initiation and 147 days for 50 per cent flowering. Whereas,

days for bud initiation and 50 per cent flowering in diploid plants were 112 and 130 respectively. Days taken by bud for full development into flower in C_0 plant and diploid plants were 14 and 12 respectively and days between pod initiation to maturity were 36 and 38 in the diploid and induced tetraploid respectively. Days to 50 per cent pod maturity were observed to be 192 and 205 in diploid and C_0 plant respectively.

In C_1 plants, days from sowing to bud initiation ranged from 108 to 121 and days from sowing to flowering ranged from 130 to 143. The days taken by bud for full development into flower and days from pod initiation to maturity ranged from 12 to 15 and 34 to 40 respectively. Days to 50 per cent pod maturity ranged from 200 to 220 in these C_1 plants.

3. Leaf:

The leaves of C_0 plant were thicker and darker green in colour. An increase in breadth and decrease in length of leaves in C_0 plants (Table-119) was noticed in comparison to its diploid as the leaf length and breadth in C_0 plant was 4.5 cm and 2.4 cm respectively as against 4.8 cm and 2.0 cm in diploid. Reduction in petiolar length was observed in C_0 plant as it was 2.0 cm in induced tetraploid and 2.30 cm in the diploid plants. The surface of leaves was also more hairy in tetraploid as compared to those of dioloids.

In C_1 generation, length and breadth of induced tetraploid plants ranged from 4.6 cm to 5.8 cm and 2.5 to 2.9 cm respectively. Petiolar length in these C_1 plants ranged from 2.1 to 2.8 cm, the average being 2.6 cm. The leaves of C_1 plants were also thicker and darker green in colour. In all the C_1 plants, the leaves were densely hairy.

4. Flower:

The C_0 plant produced larger flowers as compared to those of diploid. The size of standard petal of C_0 plant was 2.88 cm^2 as against 2.30 cm^2 in diploid. Similarly the length of style was also increased in the tetraploid as it was 1.8 cm in C_0 and 1.6 cm in diploid.

In C_1 plants, the size of the standard petal ranged from 2.88 to 2.92 cm^2 , the average being 2.89 cm^2 . Increase in stylor length was also observed as it ranged from 1.7 to 1.9, the average being 1.9 cm (Table 120).

5. Pod setting:

The induced tetraploid (C_0) of A. lineata showed 16.66 per cent pod setting as against 63.50 per cent in the diploid. In C_1 it ranged from 9.4 to 29.2 per cent, the average being 21.0 pod setting.

6. Pod:

An increase in pod breadth and decrease in pod length was observed in C_0 plant of A. lineata as length of tetraploid and diploid pods was 1.3 cm and 1.7 cm respectively. While breadth of pods of tetraploid and diploid was 0.8 cm and 0.6 cm respectively. Thus a slight difference in pod size of tetraploid and diploid was observed as it was 1.04 cm^2 in C_0 plant and 1.19 cm^2 in diploid. The pods of tetraploid (C_0) plant were more hairy as compared to diploid. Number of chambers per pod and number of seeds per pod was observed to be 1.6 and 0.6; 1.9 and 1.5 in induced tetraploid (C_0) and diploid respectively. A marked reduction in seed per pod was observed in induced tetraploid. Thickness of pod was 0.50 cm in tetraploid and 0.450 cm in diploid.

In C_1 plants pod sizes ranged from 0.9 to 1.52 cm², the average being 1.20 cm² pod size. Thickness of pod ranged from 0.40 cm to 0.55 cm, the average being 0.50 cm. In these plants, number of chambers per pod ranged from 1-2 and the number of seeds per pod ranged from 0.5 to 2.0, the average being 1.00 seeds per pod. All the pods of C_1 plants studied, showed short and dense hair.

6. Ovule fertility:

Percentage fertility of ovule was 27.27 and 85.0 in induced tetraploid (C_0) and the diploid respectively. In C_1 plants it ranged from 32.0 to 59.0, the average being 41.0 per cent ovule fertility.

7. Seeds:

The seeds of C_0 plant were thicker and more bold in comparison to diploid. Average thickness of seed was 0.40 cm and 0.34 cm in tetraploid and diploid respectively. In C_1 plants, seed thickness ranged from 0.38 cm to 0.49 cm, the average being 0.42 cm.

8. Stomata:

Marked increase in the size of stomata in tetraploid plant over the diploid was noticed. The length and breadth of stomata of C_0 plant was 18.0 u and 15.0 u respectively while it was 15 u and 12 u in diploid. However, tetraploid exhibited reduction in number of stomata per unit area (5.0) as compared to diploid (8.0).

In C_1 plants too, reduction in the number of stomata per unit area was observed and the mean value of 6.0 stomata per unit area was recorded. In these C_1 plants the size of stomata ranged from 258 μ to 283 μ , the average being 274 μ (Table-119).

Cytology (C_0).Mitosis:

Mitotic studies in root tip cells of colchicine treated seeds of A. lineata revealed different polidy leaves as $4n$, $8n$ and $16n$ (Plate-25; Figs. 14,15) (Table-120) at different concentrations and durations. 0.025% colchicine solution used for 6 hours brought about only condensation of chromosomes. While at 0.05% concentration and 6 hours duration of treatment, 3.12% cells showed chromosome doubling ($4n = 44$). In the treatment with 0.1% concentration and 6 hours duration, numerical changes in chromosomes viz., 44 and 88 were observed in 19.78% and 13.3% cells respectively. In the treatment with 0.2% colchicine applied for 2 hours, 44 chromosomes were observed in 7.14% cells and in remaining 92.85% cells 22 condensed chromosomes were observed. When 0.2% colchicine applied for 4 hours, 22 and 44 chromosomes were observed in 54.0 and 46.0 per cent cells, respectively. When 0.2% colchicine applied for 6 hours, $4n$ and $8n$ ploidy levels were observed in 81.6 and 16.8 per cent cells respectively. The highest concentration (0.2%) of colchicine solution when used for 8 hours duration resulted in 75.0% cells with $8n$ and 15.0% cells with $16n$ chromosomes.

Meiosis:

Meiotic study in C_0 plant revealed various chromosome associations as quadrivalent, trivalent, bivalent and univalent at metaphase-I (Table-121). It is clear from the Table-121 that at metaphase-I, quadrivalents ranged from 0-8 with 4.72 per cell. Trivalent, bivalent and univalents ranged from 0-1, 6-21 and 0-12 with 0.03, 12.54 and 0.93 per cell respectively. The maximum number of 8 IVs was observed in 20.64% of cells. The maximum frequency of the cells were observed with chromosomal associations of

5 IVs, + 12 IIs (Plate-19; Fig. 1) in 34.9% cells. At metaphase-I maximum number of 12 univalents was observed in 3.44% cells. Chiasma frequency as observed at metaphase-I was 40.5 per cell in C_0 plant (Table-123). At anaphase-I, unequal distribution of chromosomes to the poles (Plate-19; Fig. 10) (20: 24) was observed in 5.26% of PMCs and in remaining 94.68% cells, equal distribution of chromosomes to the poles was observed (Table-124). At sporad stage tetrads, polyads (Plate-19; Fig. 5) and micronuclei were observed in 92.22%, 3.33% and 4.44% cells respectively (Table-124).

Reduction in pollen fertility was observed as compared to diploids as it was 82.5% in induced tetraploid and % in the diploid. Marked increase in pollen size was observed in tetraploid plant. Fertile pollen size ranged from 36 μ to 48 μ with 43.6 μ mean diameter (Plate-19; Fig. 6,7). A slight reduction in the number of pollen grains per microscopic field was also observed. In diploids fertile pollen size ranged from 36-42 μ .

Cytology: (C_1).

a) Mitosis:

Mitotic study of C_1 plant revealed $4n = 44$ (Plate-19; Fig. 11) at somatic metaphase of root tip cells.

b) Meiosis:

Meiotic studies were carried out in 2 tetraploid plants separately.

Plant No. 1:

Data on chromosomal associations revealed pollen grain mother cells with varying number of quadrivalents,

bivalents and univalents at metaphase-I. In this plant quadrivalents ranged from 0-5 with 2.47 per cell. And maximum number of 5 IVs were observed in 34.05% cells. Bivalents and univalents, at metaphase-I, ranged from 12-22, and 0-44 with 13.2 and 2.36 per cell respectively (Table-122). Maximum number of 22 bivalents (Plate-19; Fig. 8) were noticed in 22.70% cells. Maximum number of 44 univalents (Plate-19; Fig. 3) were observed in 4.54% cells. Chiasma frequency as observed at metaphase-I, was 34.22 per cell in this C_1 plant (Table-123). At anaphase-I, unequal distribution of chromosomes to the poles was observed in 2.22% cells and in remaining 97.7% cells equal separation was observed. At sporad stage tetrads, polyads and micronuclei were observed in 96.47% and 2.35% cells respectively.

Pollen fertility percentage was 84.7 and fertile pollen size ranged from 42 u to 48 u with 46.5 u mean diameter (Table-124).

Plant No. 2:

The data on chromosomal associations in this C_1 plant also indicated varying number of quadrivalents and bivalents at metaphase-I (Table-122). There were 6 IVs in 26.20% cell. And the maximum frequency of cells observed with chromosomal association of 6 IVs + 10 IIs (26.20%) (Table-122). At metaphase-I, quadrivalents ranged from 0-6 with 3.68 per cell while bivalents and univalents ranged from 10-22 and 0-4 with 14.49 and 0.36 per cell. Maximum number of 22 bivalents were observed in 13.10% cells and maximum four univalents (Plate-19; Fig. 9) were observed in 6.55% cells. Four quadrivalents (Plate-19; Fig. 2) were observed in 13.10% cells at metaphase-I. Chiasma frequency as observed at metaphase-I was 42.34 per cell (Table-123). At anaphase-I unequal distribution of chromosomes to the

effects of colchicine on seed germination and plant survival in Atriplex linearis (JM 2639)

| Seed Treatment | | | Seedling treatment (immersion) | | | Seedling treatment (drop through cotton plug method) | | |
|-------------------|-----------------|------------------|--------------------------------|----------|---------------------------|--|-----------------|--------------------|
| Concentration (%) | Duration (hrs.) | Seeds germinated | Concentration (%) | Duration | No. of seedlings survived | Concentration (%) | Duration (hrs.) | Seedlings survived |
| 0.05 | 4 | 10 (100) | 0.05 | 4 | 10 (100) | 0.05 | A ₁ | 10 (100) |
| 0.05 | 6 | 10 (100) | 0.05 | 6 | 10 (100) | 0.05 | A ₂ | 10 (100) |
| 0.05 | 8 | 10 (100) | 0.05 | 8 | 10 (100) | 0.05 | A ₃ | 10 (100) |
| 0.05 | 24 | 1 (10) | 0.05 | 1 | 1 (10) | 0.05 | A ₁ | 10 (100) |
| 0.1 | 4 | 10 (100) | 0.1 | 4 | 20 (200) | 0.1 | A ₁ | 20 (95.0) |
| 0.1 | 6 | 10 (100) | 0.1 | 6 | 20 (200) | 0.1 | A ₂ | 20 (90) |
| 0.1 | 8 | 10 (100) | 0.1 | 8 | 20 (200) | 0.1 | A ₃ | 20 (85.0) |
| 0.1 | 24 | 6 (60.0) | 0.2 | 2 | 20 (200) | 0.2 | A ₁ | 20 (80) |
| 0.2 | 2 | 9 (90.0) | 0.2 | 4 | 20 (200) | 0.2 | A ₂ | 20 (55) |
| 0.2 | 4 | 9 (90.0) | 0.2 | 6 | 30 (300) | 0.2 | A ₃ | 2 (4.0) |
| 0.2 | 6 | 9 (90.0) | 0.2 | 8 | 20 (200) | 0.2 | | |
| 0.2 | 8 | 7 (70.0) | 0.2 | | | | | |

No. of seeds treated in each case = 10.

Table - 119

Comparative morphology of diploid and induced tetraploids of Atylosia lineata (JM 2639)

| Characters | <u>A. lineata</u> | | <u>A. lineata</u> | | <u>A. lineata</u> | |
|---|-------------------|--|----------------------|--|----------------------|--|
| | 2x | | 4x (C ₀) | | 4x (C ₁) | |
| No. of primary branches | 5 | | 2 | | 6 | |
| No. of secondary branches | 8 | | 3 | | 7 | |
| Central leaflet: surface | Hairy + | | Hairy ++ | | Hairy ++ | |
| (L x B) cm. | 4.8 x 2.0 | | 4.5 x 2.4 | | 5.2 x 2.6 | |
| length of petiole (cm.) | 2.30 | | 2.00 | | 2.6 | |
| height of plant (cm.) | 110 | | 56.0 | | 116 | |
| Days from sowing to bud initiation | 112 | | 127 | | 115 | |
| Days from sowing to flowering | 130 | | 147 | | 135 | |
| Days between bud to flower | 12 | | 14 | | 13 | |
| Days between pod initiation to maturity | 36 | | 38 | | 35 | |
| Size of the standard petal (L x B) cm. | 1.6 x 1.5 | | 1.8 x 1.6 | | 1.79 x 1.62 | |
| Length of style (cm.) | 1.60 | | 1.80 | | 1.79 | |
| pod (L x B) cm. | 1.7 x 0.7 | | 1.3 x 0.8 | | 1.5 x 0.8 | |
| Thickness of pod (cm.) | 0.450 | | 0.500 | | 0.500 | |
| Hairs on mature pod | Present | | Present | | Present | |
| Thickness of seed (cm.) | 0.34 | | 0.400 | | 0.420 | |
| No. of chambers per pod | 1.9 | | 1.6 | | 1.8 | |
| No. of seeds per pod | 1.5 | | 0.6 | | 1.00 | |
| Days to maturity | 192 | | 208 | | 210 | |
| Pod set (%) | 63.5 | | 16.66 | | 21.00 | |
| Ovule fertility (%) | 85.0 | | 27.27 | | 41.0 | |
| Stonata, frequency (L x B) μ | 8.0 | | 5.0 | | 6.0 | |
| | 15.0 x 12.0 | | 18.0 x 15.0 | | 18.2 x 15.1 | |

12
60
23

Table - 120
Effects of colchicine on somatic chromosomes of Atylosia lineata (JM 2639)
(% in parentheses)

| Concentration (%) | Duration (hours) | No. of cells studied | PLOIDY LEVEL AT METAPHASE | | | |
|-------------------|------------------|----------------------|---------------------------|--------------|--------------|-------------|
| | | | 2n | 4n | 8n | 16n |
| 0.025 | 6 | 25 | 25 (100) | - | - | - |
| 0.05 | 6 | 32 | 31 (96.72) | 1 (3.12) | - | - |
| 0.1 | 6 | 30 | 20 (66.6) | 6 (19.98) | 4 (13.3) | - |
| 0.2 | 2 | 28 | 26 (92.85) | 2 (7.14) | - | - |
| 0.2 | 4 | 33 | 18 (54.0) | 15 (45.0) | - | - |
| 0.2 | 6 | 41 | - | 34 (81.6) | 7 (16.8) | - |
| 0.2 | 8 | 40 | - | 4 (10.0) | 30 (75.0) | 6 (15.0) |

Table - 121

Chromosome associations at Metaphase - I in Atylosia
lineata (JM 2639)

| No. of cells studied | Chromosome association at M - I | | | | Frequency Per cent | |
|----------------------------|------------------------------------|------|-------|------|--------------------|-------|
| | IV | III | II | I | | |
| 58 | 8 | - | 6 | - | 12 | 20.64 |
| | 6 | - | 9 | 2 | 8 | 13.79 |
| | 5 | 1 | 8 | 1 | 2 | 3.44 |
| | 5 | - | 12 | - | 20 | 34.4 |
| | 3 | - | 16 | - | 4 | 6.89 |
| | 2 | - | 18 | - | 4 | 6.89 |
| | - | - | 16 | 12 | 2 | 3.44 |
| | - | - | 21 | 2 | 6 | 10.32 |
| Range | 0-8 | 0-1 | 6-21 | 0-12 | | |
| Mean | 4.72 | 0.03 | 12.54 | 0.93 | | |

Table - 122

Chromosome associations at Metaphase - I in induced tetraploid of Atylosia lineata (C₁)

| Plant No. | No. of cells studied | Chromosome associations at M-I | | | | Frequency | Per cent |
|-----------|----------------------|--------------------------------|-----|-------|------|-----------|----------|
| | | IV | III | II | I | | |
| 1 | 44 | 5 | - | 12 | - | 15 | 34.05 |
| | | 3 | - | 16 | - | 8 | 18.18 |
| | | 2 | - | 18 | - | 3 | 6.81 |
| | | 2 | - | 16 | 4 | 2 | 4.54 |
| | | - | - | 22 | - | 10 | 22.70 |
| | | - | - | 20 | 2 | 4 | 9.09 |
| | | - | - | - | 44 | 2 | 4.54 |
| Range | | 0-5 | - | 12-22 | 0-44 | | |
| Mean | | 2.47 | - | 13.2 | 2.30 | | |
| 2 | 61 | 6 | - | 10 | - | 16 | 26.20 |
| | | 5 | - | 12 | - | 14 | 22.95 |
| | | 4 | - | 14 | - | 8 | 13.10 |
| | | 3 | - | 16 | - | 5 | 8.15 |
| | | 2 | - | 18 | - | 6 | 9.78 |
| | | - | - | 22 | - | 8 | 13.10 |
| | | - | - | 20 | 4 | 4 | 6.55 |
| Range | | 0-6 | - | 10-22 | 0-4 | | |
| Mean | | 3.68 | - | 14.49 | 0.36 | | |

Table - 123

Chiasma frequency at M-I in induced tetraploid of Atylosia lineata

| Generations | No. of cells studied | Quadrivalents with 3xmata | No. of trivalents | No. of bivalents with 2xmata | 1xmata | No. of univalents | Total xmata per cell | | |
|----------------------------|----------------------|---------------------------|-------------------|------------------------------|--------|-------------------|----------------------|------|-------|
| C ₀ | 58 | 74 | 199 | 2 | 600 | 127 | 54 | 2349 | 40.5 |
| C ₁ Plant No. 1 | 44 | 8 | 101 | - | 497 | 84 | 104 | 1506 | 34.22 |
| Plant No. 2 | 61 | 5 | 220 | - | 804 | 80 | 16 | 2583 | 42.34 |

Table - 124

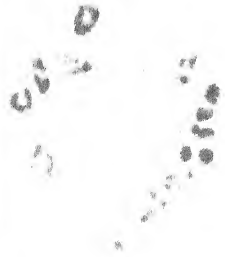
Chromosome distribution at Anaphase - I in induced tetraploid of Atylosia lineata (JM 2639) (% in parentheses)

| Generation | No. of cells studied | Normal separation | Unequal distribution | No. of cells studied | Tetrad | Sporad Stage | Pollen fertility | Fertile pollen size Range (μ) | Mean (μ) |
|----------------------------|----------------------|-------------------|----------------------|----------------------|------------|--------------|------------------|-------------------------------|----------|
| C ₀ | 38 | 36 (94.68) | 2 (5.26) | 90 | 83 (92.22) | 3 (3.33) | 4 (4.44) | 36-48 | 43.6 |
| C ₁ Plant No. 1 | 45 | 44 (97.7) | 1 (2.22) | 85 | 82 (96.47) | 1 (1.17) | 2 (2.35) | 42-48 | 46.5 |
| Plant No. 2 | 51 | 49 (96.04) | 2 (3.92) | 93 | 92 (98.9) | - | 1 (1.07) | 39-51 | 45.7 |

PLATE - 19 (Induced tetraploid of A. lineata)

- Fig. 1. 5 IV's + 12 II's at Metaphase-I (C_0) (X1500)
- Fig. 2. 4 IV's + 14 II's at Metaphase-I (C_1) No.2 (X 1500)
- Fig. 3. 44 I's at Metaphase-I (C_1) No. 1 (X 1500)
- Fig. 4. Laggards at Anaphase-I (C_1) No. 2.
- Fig. 5. Hexad with normal tetrad (C_0)
- Fig. 6. Pollen grains of diploid (X 400)
- Fig. 7. Pollen grains of tetraploid (X 400)
- Fig. 8. 22 bivalents at Metaphase-I (C_1) No. 1 (X 1500)
- Fig. 9. 20 II's + 4 I's at Metaphase-I (C_1) No. 1 (X 1500)
- Fig. 10. Unequal separation of chromosome at Anaphase-I (24-20) (C_0) (X 1500)
- Fig. 11. 44 somatic chromosomes at Metaphase (C_1) (X 1500)

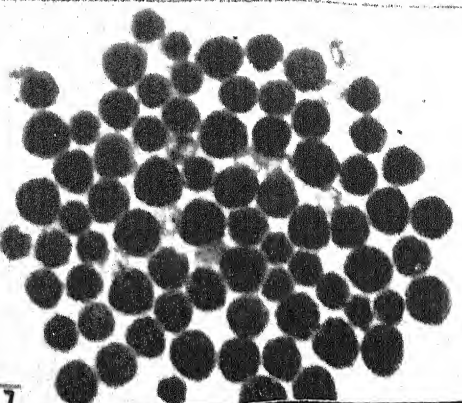
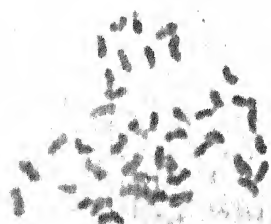
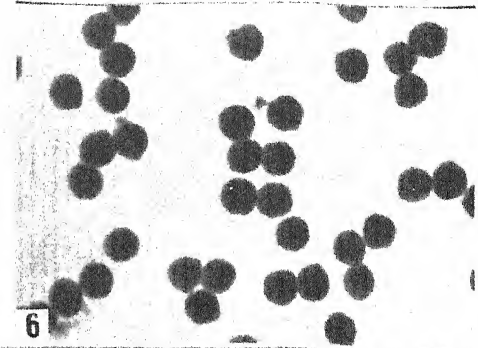
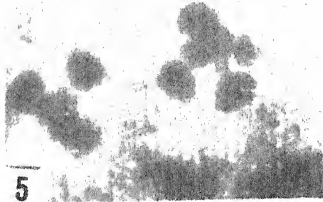
PLATE - 19



1



3



poles (Plate-19; Fig. 4) was observed in 3.92% cells and in remaining 96.04% cells equal separation of chromosomes to the pole was observed (Table-124). At sporad stage, tetrads and micronuclei were observed in 98.9% and 1.07 cells respectively.

Pollen fertility was 86.2%. Fertile pollen size ranged from 39 μ to 51 μ with 45.7 μ mean diameter (Table-124).

Observations on the effect of colchicine in *Atylosia cajanifolia*.

a) Seed germination:

The effects of colchicine on seed germination at different concentrations and duration (Table-125) are as follows:

In the treatment with 0.05% colchicine applied for 4, 6 and 8 hours, germination of all the seeds was recorded. When 0.05% colchicine applied for 24 hours, 60 per cent seed germination was observed. In the treatment with 0.1% colchicine applied for 4, 6, 8 and 24 hours, seed germination was 100, 100, 90 and 40 per cent respectively. In the treatment with 0.2% colchicine applied for 2, 4, 6 and 8 hours, 90, 90, 70 and 30 per cent seed germination was recorded. The time taken by treated seeds for germination varied from 2-4 days while the untreated seeds germinated in 1-3 days.

Plant survival:

The effects of colchicine on plant survival was studied in seed and seedling treatments (Table-125). Survival percentage differed in both the treatments.

In seed treatment, plant survival varied from 0-20%. The highest (20%) having been recorded with 0.05% colchicine applied for 4 hours. In the treatment with 0.05% for 6 hours, 10 per cent plants survived and when 0.05% colchicine applied for 8 and 24 hours, plants could not be survived. In the treatment with 0.1% colchicine applied for 4 hours, 10 per cent plants survival was recorded, and in the treatment with 0.1% colchicine applied for 6, 8 and 24 hours, plants could not be survived. In the treatment with 0.2% colchicine applied for 2, 4, 6 and 8 hours, plants could not survive. The seed treatment was not successful, main cause of failure appeared to be the drastic effect of colchicine on roots, which failed to produce lateral roots and hence seedling could not develop properly after respective treatments. The plants which survived after the treatments were also found to be diploid on their cytological examination.

When seedlings were immersed in 0.05% aqueous colchicine solution for 4, 6 and 8 hours, percentage survival observed was 80.0, 70.0 and 60.0 respectively. When seedlings were immersed in 0.1% colchicine solution for 4 and 6 hours, 26.6, and 6.66 per cent seedlings survived respectively. 0.1% colchicine when applied for 8 hours, seedlings could not survive. When seedlings were immersed in 0.2% colchicine solution for 2 hours, 15.0 per cent seedlings survived and when the seedlings were immersed in 0.2% colchicine for 4, 6 and 8 hours, they could not survive.

Colchicine treatment of seedlings through absorbent cotton plug soaking method exhibited differential survival of seedlings at different concentrations and durations.

When 0.05% colchicine applied for 8 hours a days for one, two and three days, all the seedlings survived. In the treatment with 0.1% colchicine applied for 8 hours a day for one, two and three days, percentage survival of seedling was 90.0, 85.0 and 70.0 respectively. When 0.2% colchicine applied for 8 hours a day for one and two days, seedling survival percentage was 60.0 and 15.0 respectively. When 0.2% colchicine applied for 8 hours a day for 3 days seedlings could not survive.

Production of polyploids:

In the seed treatment with 0.05% and 0.1% colchicine applied for 4, 6, 8 and 24 hours and 0.2% for 2, 4, 6 and 8 hours polyploids plants could not be obtained.

Similarly, in the treatments with 0.05% and 0.1% colchicine for 4, 6 and 8 hours, tetraploid plants could not be obtained. However, chromosome doubling was successfully induced through the apical bud treatments wherein colchicine solution 0.2% strength was applied for 8 hours a day for 2 days.

Studies on induced tetraploid of *A. caianifolia*.

a) Morphology:

Comparative morphological characters of diploid and induced tetraploids of *A. caianifolia* are summarised in Table-126. Detail observations pertaining to the morphology of diploid and induced tetraploid of *A. caianifolia* are presented here.

1. Seedling, branches and stem height:

Immediately after treatment of the apical buds of the seedlings, the first pair of leaves gradually became

darker green in colour and thicker than the untreated ones. The induced tetraploid plant of A. caianifolia showed less number of primary and secondary branches in comparison to its diploid counterparts. The number of primary and secondary branches in diploid and tetraploid plants were 5 and 7; 3 and 5 respectively. Tetraploid plant showed reduced height (121 cm) as compared to diploid (127 cm). Stem of induced tetraploid was thicker with shorter internodes in comparison to its diploid.

In the C_1 generation first pair of simple leaves were darker green in colour and thicker than their diploids. In C_1 plants, the number of primary and secondary branches ranged from 5 to 9 and 6 to 12 respectively and stem height ranged from 120 to 135 cm, the average being 125 cm.

2. Days to flowering and maturity:

Delayed flowering and maturity were observed in tetraploids in contrast to diploids.

The C_0 plant of induced tetraploid taken 130 days for bud initiation and 151 days for 50 per cent flowering. whereas, days for bud initiation and 50 per cent flowering in diploid plants were 110 and 130 days respectively. Days taken by bud for full development into flower in C_0 plants and diploid plants were 16 and 12 respectively and days between pod initiation to maturity were 41 and 33 in the induced tetraploid and diploid respectively. Days to 50 per cent pod maturity were observed to be 227 and 197 in C_0 plant and their diploid respectively.

In C_1 plants, days from sowing to bud initiation ranged from 118 to 132 and days from sowing to flowering ranged from 140 to 159. The days taken by bud for full development into flower and days between pod initiation to maturity ranged from 13 to 17 and 32 to 40 days respectively. Days to 50 per cent pod maturity ranged from 200 to 220 in these C_1 plants.

3. Leaf:

The leaves of C_0 plants were thicker and darker green in colour. An increase in length and breadth of leaves in C_0 plant (Plate-20; Fig. 1) was noticed in comparison to its diploid counter part. The leaf length and breadth in C_0 plant was 5.3 cm and 2.4 cm respectively as against 4.8 cm and 2.0 cm in diploid. Petiolar length was observed to be slightly increased in the induced tetraploid as it was 1.90 cm in C_0 plant and 1.70 cm in the diploid. The surface of leaves was also more hairy as compared to those of diploids.

In C_1 generation, length and breadth of induced tetraploid plants ranged from 5.0 to 8.5 cm and 2.0 to 3.6 cm respectively. The leaves of C_0 plants were also thicker and darker green in colour. The leaves of C_1 plants showed vigour in length and breadth, over diploid as well as C_0 plant. Petiolar length in these C_1 plants ranged from 1.6 to 2.2 cm, the average being 2.0 cm. In all the C_1 plants, the leaves were densely hairy.

4. Flower:

The tetraploid plant (C_0) produced larger flower as compared to those of diploid (Plate-20; Fig. 2). The size of standard petal of C_0 plant was 3.8 cm^2 as against 2.56 cm^2 in diploid. Similarly, the length of style was also increased in the tetraploid (1.9 cm in tetraploid and 1.6 cm in diploid.).

In C_1 plants, size of the standard petal ranged from 3.61 to 4.8 cm^2 , the average being 3.99 cm^2 . Increase in stylor length was also observed. It ranged from 1.8 to 2.1 cm, the average being 1.9 cm.

5. Pod setting:

The induced tetraploid (C_0) of Alylosia caianifolia showed 12.5 per cent pod setting as against 38.0 per cent pod setting in the diploid. In C_1 it ranged from 15.2 to 22.5 per cent, the average being 18.0 per cent pod setting.

6. Pod:

A slight reduction in pod size was observed in C_0 plant as compared to diploid. The size of pods were 2.52 and 2.32 cm^2 in diploid and C_0 tetraploid respectively. Thickness of pod was more in tetraploid as it was 0.60 cm in tetraploid as against 0.50 cm in diploid. The pods were more hairy in case of tetraploid plant as compared to diploid. A marked difference in the number of seeds per pod was noticed (Table-126), as it was 2.50 in diploid and 0.50 in tetraploid.

In C_1 plants, pod size ranged from 2.24 to 3.24 cm^2 , the average being 2.48 cm^2 . Thickness of pods ranged from 0.56 cm to 0.63 cm, the average being 0.60 cm. The these plants, number of chambers per pod ranged from 2 to 3 and the number of seeds ranged from 0.7 to 1.9, the average being 1.13 seeds per pod. All the pods of C_1 plants studied, showed short and dense hair.

6. Ovule fertility:

Percentage fertility of ovule was 18.51 and 90.5 in induced C_0 plant and the diploid respectively. In C_1 plants it ranged from 28.6 to 51.2 per cent, the average

being 36.21 per cent.

7. Seed:

The seeds of C_0 plant were thicker and more bold in comparison to diploid. Average thickness of seed was 0.40 cm and 0.50 cm in diploid and tetraploid respectively. In C_1 plants, seed thickness ranged from 0.50 to 0.60 cm, the average being 0.55 cm seed thickness.

8. Stomata:

Marked increase in the size of stomata in tetraploid plant over the diploid was noticed. The length and breadth of stomata was 21 μ and 18 μ in tetraploid while it was 18 μ and 15 μ in diploid. However, tetraploid exhibited lesser number of stomata (5.0) per unit area as compared to diploid (7.0).

In C_1 plants too, reduction in the number of stomata per unit area was observed and the mean value of 4.8 stomata per unit area was recorded. In these C_1 plants, the size of stomata ranged from 318 μ to 378 μ , the average being 355.6 μ (Table-126).

b) Cytology:

Mitosis: (C_0).

Mitotic studies in root tip cells of colchicine treated seeds of A. caianifolia revealed different ploidy levels as 4n, 8n, and 16n (Plate-24; Figs.5,7) (Table-127) at different concentrations and durations. 0.25% colchicine solution when used for 6 hours brought about only condensation of chromosomes. While at 0.05% concentration and 6 hours duration of treatment, 6.6 per cent cells showed chromosome doubling i.e. 4n = 44. In the treatment with 0.1% concentration and 6 hours duration, numerical

changes in chromosomes viz., 44 and 88 were observed in 35.0 per cent and 15.0 per cent dividing cells respectively. In the treatment with 0.2% colchicine applied for 2 hours, 44 chromosomes were observed in 20.0 per cent cells and in remaining 80.0 per cent cells, 22 condensed chromosomes were observed. When 0.2% colchicine applied for 4 hours, 22 and 44 chromosomes were observed in 30 and 70 per cent cells respectively. When 0.2% colchicine applied for 6 hours, 4n, 8n and 16n ploidy levels were observed in 84.0, 11.4 and 2.85 per cent cells respectively. The highest concentration of 0.2% colchicine when applied for 8 hours, increase in percentage of cells having more than 44 chromosomes was observed (48.0% cells with 8n chromosomes and 12.8% cells with 16n chromosomes). The treatment of 0.2% concentration for 6 and 8 hours duration brought all polyploid cells.

Meiosis (C_0).

Meiotic study in C_0 plant revealed various chromosomal associations as quadrivalent, trivalent, bivalent and univalent at metaphase-I (Table-128). It can be seen from the Table-128, that at metaphase-I, quadrivalents ranged from 0-11 with 4.31 per cell. Trivalent, bivalent and univalent ranged from 0-1, 0-20 and 0-6 with 0.13, 11.5 and 0.66 per cell respectively. The maximum number of 11 IVs (Plate-20; Fig. 3) was observed in 19.98 per cent cells and 10 IVs + 2 IIs (Plate-20; Fig. 4) in 6.66 per cent cells at metaphase-I. The maximum number of 6 univalents was observed in 2.22 per cent cells. Chiasma frequency as observed at metaphase-I was 39.8 per cell (Table-130). At anaphase-I, unequal distribution of chromosomes to the poles (21:23 and 20:24) was observed in 2.0 and 4.0 per cent cells respectively. However, in the remaining 94.0 per cent cells, equal separation of chromosomes to the poles was observed (Table-131). At spored stage tetrads and

polyads were observed in 88.92 and 3.52 per cent cells respectively and in 7.02 per cent cells micronuclei were noticed (Table-131).

In the induced tetraploid pollen fertility percentage was 86.2 and fertile pollen size ranged from 42-51 μ with 48.2 μ mean diameter (Plate-20; Fig. 10). Thus a marked increase in the size of fertile pollen grains was observed as compared to its diploid counterpart where pollen size ranged from 36-42 μ . A slight reduction in the number of pollen grain per unit area was also noticed.

Cytology (C_1).

a) Mitosis:

Mitotic study of C_1 plants revealed $4n = 44$ (Plate-20; Fig. 11) at somatic metaphase of root tip cells.

b) Meiosis:

Meiotic studies were carried out in 3 tetraploid plants separately.

Plant No. 1:

Data on chromosomal associations revealed pollen grain mother cells with varying number of quadrivalents and bivalents at metaphase-I. In this plant, at metaphase-I, 32.5 per cent cells with 6 quadrivalents, 30.0 per cent cells with 5 IVs and 7.5 per cent cells with 3 IVs were observed (Table-129). A range of quadrivalents from 3-6 with 3.85 per cell was recorded. Bivalents ranged from 0-22 with 13.45 per cell and univalents ranged from 0-44 with 2.4 per cell. Maximum (32.5%) cells showed chromosomal association of 6 IVs + 10 IIs (Table-129). Maximum number of 22 IIs were observed in 20.0 per cent cells (Plate-20; Fig. 6) and formation of 44 univalents were recorded in

5.0 per cent of PMCs. Chiasma frequency as observed at metaphase-I was 40.25 per cell (Table-130). At anaphase-I, unequal distribution of chromosomes to the poles was observed in 3.07 per cent cells and equal separation of chromosomes in the remaining cells. At the sporad stage, polyads (Plate-20; Fig. 7,8) and micronuclei were observed in 3.33 and 1.11 per cent cells respectively. In remaining cells tetrad formation was registered (Table-131). Pollen fertility was 87.6 per cent, and fertile pollen size ranged from 39 μ to 51 μ with 46.8 μ mean diameter.

Plant No. 2:

The data on chromosomal associations in autotetraploid also indicated varying number of quadrivalents and bivalents in different PMCs (Table-129). There were 7 IVs in 15.36 per cent cells and the maximum number of cells showed 6 IVs + 10 II (38.40%). Quadrivalents ranged from 2-7 with 5.43 per cell and bivalents from 8-18 with 12.46 per cell. Chiasma frequency was 40.25 per cell at metaphase-I (Table-130). At anaphase-I unequal distribution of chromosomes to the poles was observed in 3.92 per cent cells and in remaining 94.08 per cent cells, equal separation was observed. At sporad stage, formation of tetrads polyads and micronuclei was observed in 95.28, 1.90 and 3.80 per cent cells respectively. Pollen fertility was 92.0 per cent and fertile pollen size ranged from 42-51 with 49.2 μ mean diameter.

Plant No. 3:

In this plants, meiotic study revealed quadrivalents, bivalents and univalents (Plate-20; Fig. 5) (Table-129). At metaphase-I, quadrivalents ranged from 0-6 with 4.81 per cell and bivalents ranged from 9-22 with 11.00 per cell.

Table - 125

Effect of colchicine on seed germination and plant survival in *Alysicarpus esenifolia*. No. of seeds treated in each case were = 10.

| Seed treatment | | Seedling treatment (immersion) | | Seedling treatment (drop through cotton method) | | No. of seedlings treated | No. of seedlings survived | Survival (%) | Days | Concn. (%) | Days | Survival (%) | No. of seedlings treated | No. of seedlings survived | Survival (%) | Days | Concn. (%) | Days | Survival (%) | No. of seedlings treated | No. of seedlings survived | Survival (%) |
|----------------|-------------------|--------------------------------|-------------------|---|-------------------|--------------------------|---------------------------|--------------|------|------------|----------------|--------------|--------------------------|---------------------------|--------------|------|------------|------|--------------|--------------------------|---------------------------|--------------|
| Concn. (%) | Dura- tion (hrs.) | Seeds germinated | Seedling survived | Concn. (%) | Dura- tion (hrs.) | Seedlings treated | Seedlings survived | Survival (%) | Days | Concn. (%) | Days | Survival (%) | Seedlings treated | Seedlings survived | Survival (%) | Days | Concn. (%) | Days | Survival (%) | Seedlings treated | Seedlings survived | Survival (%) |
| 0.05 | 4 | 10 (100) | 2 (20.0) | 0.05 | 4 | 10 | 8 (80.0) | | | 0.05 | A ₁ | 10 (100) | 10 | 10 (100) | 0 | | | | | 10 | 10 (100) | 0 |
| 0.05 | 6 | 10 (100) | 1 (10.0) | 0.05 | 6 | 10 | 7 (70.0) | | | 0.05 | A ₂ | 10 (100) | 10 | 10 (100) | 0 | | | | | 10 | 10 (100) | 0 |
| 0.05 | 8 | 10 (100) | 0 | 0.05 | 8 | 10 | 6 (60.0) | | | 0.05 | A ₃ | 10 (100) | 10 | 10 (100) | 0 | | | | | 10 | 10 (100) | 0 |
| 0.05 | 24 | 6 (60) | 0 | 0.05 | 24 | 6 | 0 | | | 0.05 | A ₁ | 6 (60) | 6 | 6 (100) | 0 | | | | | 6 | 6 (100) | 0 |
| 0.1 | 4 | 10 (100) | 1 (10) | 0.1 | 4 | 15 | 4 (26.6) | | | 0.1 | A ₁ | 15 (100) | 15 | 15 (100) | 0 | | | | | 15 | 15 (100) | 0 |
| 0.1 | 6 | 10 (100) | 0 | 0.1 | 6 | 15 | 1 (6.66) | | | 0.1 | A ₂ | 15 (100) | 15 | 15 (100) | 0 | | | | | 15 | 15 (100) | 0 |
| 0.1 | 8 | 9 (90.0) | 0 | 0.1 | 8 | 15 | 0 | | | 0.1 | A ₃ | 15 (100) | 15 | 15 (100) | 0 | | | | | 15 | 15 (100) | 0 |
| 0.1 | 24 | 4 (40) | 0 | 0.1 | 24 | 4 | 0 | | | 0.1 | A ₁ | 4 (40) | 4 | 4 (100) | 0 | | | | | 4 | 4 (100) | 0 |
| 0.2 | 2 | 9 (90.0) | 0 | 0.2 | 2 | 20 | 3 (15.0) | | | 0.2 | A ₁ | 20 (100) | 20 | 20 (100) | 0 | | | | | 20 | 20 (100) | 0 |
| 0.2 | 4 | 9 (90) | 0 | 0.2 | 4 | 20 | 0 | | | 0.2 | A ₂ | 20 (100) | 20 | 20 (100) | 0 | | | | | 20 | 20 (100) | 0 |
| 0.2 | 6 | 7 (70.0) | 0 | 0.2 | 6 | 20 | 0 | | | 0.2 | A ₃ | 20 (100) | 20 | 20 (100) | 0 | | | | | 20 | 20 (100) | 0 |
| 0.2 | 8 | 3 (30) | 0 | 0.2 | 8 | 20 | 0 | | | 0.2 | A ₁ | 20 (100) | 20 | 20 (100) | 0 | | | | | 20 | 20 (100) | 0 |

(% in parentheses)

A₁ = 8 hrs. - One day; A₂ = 8 hrs. - Two days; A₃ = 8 hrs. - 3 days.

Table - 126

Comparative morphology of diploid and induced tetraploid of Atylosia sajanifolia plants

| Characters | <u>A. sajanifolia</u> | | <u>A. sajanifolia</u> | |
|---|-----------------------|-----------------------|-----------------------|-----------------------|
| | 2x | 4x (C ₀) | 4x (C ₁) | |
| No. of primary branches | 5 | 3 | 5 | 6.5 |
| No. of secondary branches | 7 | 5 | | 8.0 |
| Central leaf-let: surface | Hairy + 4.8 x 2.0 | Hairy ++ 5.3 x 2.4 | Hairy ++ 5.3 x 2.4 | Hairy ++ 6.5 x 2.8 |
| (L x B) cm. | 1.70 | 1.90 | 1.90 | 2.0 |
| length of petiole | 110 | 130 | 130 | 125 |
| Days from sowing to bud initiation | 123 | 151 | 151 | 150 |
| Days to flowering | 12 | 16 | 16 | 14 |
| Days between bud to flower | 33 | 41 | 41 | 35 |
| Days between pod initiation to maturity | 1.27 | 1.21 | 1.21 | 1.25 |
| Height of plant (cm) | 1.6 x 1.6 | 2.0 x 1.9 | 2.0 x 1.9 | 2.1 x 1.9 |
| Size of the standard petal (L x B) cm. | 1.6 | 1.9 | 1.9 | 1.9 |
| Length of style (cm.) | 3.6 x 0.70 | 2.9 x 0.8 | 2.9 x 0.8 | 3.0 x 0.8 |
| Pod (L x B) cm. | 0.500 | 0.600 | 0.600 | 0.600 |
| Thickness of pod (cm.) | Present | Present | Present | Present |
| Hairs on mature pod | 2.61 | 2.1 | 2.1 | 2.3 |
| No. of chambers per pod | 2.50 | 0.5 | 0.5 | 1.13 |
| No. of seeds per pod | 0.400 | 0.500 | 0.500 | 0.55 |
| Thickness of seed (cm) | 197 | 227 | 227 | 208 |
| Days to pod maturity | 38.00 | 12.5 | 12.5 | 18.0 |
| Pod set (%) | 90.5 | 18.51 | 18.51 | 36.21 |
| Ovule fertility (%) | | | | |
| Stomata: | | | | |
| frequency | 7.0 | 5.0 | 5.0 | 4.8 |
| (L x B) μ | 18 x 15 | 21 x 18 | 21 x 18 | 20.2 x 17.6 |

Table - 127

Effects of colchicine on somatic chromosomes of Alysis calanifolia
(% in parentheses)

| Concen- tration (%) | Duration (Hours) | No. of cells studied | PLOIDY LEVEL AT METAPHASE | | | |
|---------------------------|---------------------|----------------------------|---------------------------|--------------|--------------|-------------|
| | | | 2n | 4n | 8n | 16n |
| 0.025 | 6 | 36 | 36 (100) | - | - | - |
| 0.05 | 6 | 30 | 28 (92.4) | 2 (6.6) | - | - |
| 0.1 | 6 | 40 | 18 (45.0) | 14 (35.0) | 8 (20.0) | - |
| 0.2 | 2 | 25 | 20 (80.0) | 5 (20.0) | - | - |
| " | 4 | 40 | 12 (30.0) | 28 (70.0) | - | - |
| " | 6 | 35 | - | 30 (84.0) | 4 (11.4) | 1 (2.85) |
| " | 8 | 31 | - | 12 (38.4) | 15 (48.0) | 4 (12.8) |

Table - 128

Chromosome association at Metaphase - I in induced
tetraploid of Atylosia cajanifolia (C₀)

| No. of cells studied | Chromosome associations at M-I | | | | Frequency | Per cent |
|----------------------------|--------------------------------|------|-------|------|-----------|----------|
| | IV | III | II | I | | |
| 45 | 11 | - | - | - | 9 | 19.98 |
| | 10 | - | 2 | - | 3 | 6.66 |
| | 9 | - | 4 | + | 2 | 4.44 |
| | 8 | - | 6 | - | 2 | 4.44 |
| | 6 | - | 10 | - | 1 | 2.22 |
| | 4 | - | 14 | - | 2 | 4.44 |
| | 3 | - | 16 | - | 2 | 4.44 |
| | 2 | - | 18 | - | 6 | 13.32 |
| | 2 | 1 | 16 | 1 | 1 | 2.22 |
| | 2 | - | 17 | 2 | 1 | 2.22 |
| | 2 | 2 | 15 | - | 1 | 2.22 |
| | 2 | - | 16 | 4 | 1 | 2.22 |
| | 1 | 1 | 18 | 1 | 1 | 2.22 |
| | 1 | - | 19 | 2 | 3 | 6.66 |
| | 1 | - | 18 | 4 | 2 | 2.22 |
| | 1 | - | 20 | - | 5 | 11.1 |
| | - | 1 | 20 | 1 | 2 | 2.22 |
| | - | - | 19 | 6 | 1 | 2.22 |
| Range | 0-11 | 0-2 | 0-20 | 0-6 | | |
| Mean | 4.31 | 0.13 | 11.52 | 0.66 | | |

Table - 129

Chromosome association at Metaphase - I in induced tetraploids of Atylosia gajanifolia (C₂)

| Plant No. | No. of cells studied | Chromosome association | | | | Frequency | Per cent |
|-----------|----------------------|------------------------|-----|-------|------|-----------|----------|
| | | IV | III | II | I | | |
| 1 | 40 | 6 | - | 10 | - | 13 | 32.5 |
| | | 5 | - | 12 | - | 12 | 30.0 |
| | | 3 | - | 16 | - | 3 | 7.5 |
| | | - | - | 22 | - | 8 | 20.0 |
| | | - | - | 20 | 4 | 2 | 5.0 |
| | | - | - | - | 44 | 2 | 5.0 |
| Range | | 0-6 | - | 0-22 | 0-44 | | |
| Mean | | 3.85 | - | 13.45 | 2.4 | | |
| 2 | 39 | 7 | - | 8 | - | 6 | 15.36 |
| | | 6 | - | 10 | - | 15 | 38.40 |
| | | 4 | - | 12 | - | 4 | 10.24 |
| | | 3 | - | 16 | - | 6 | 15.38 |
| | | 2 | - | 18 | - | 8 | 20.51 |
| | | | | | | | |
| Range | | 2-7 | - | 8-18 | - | | |
| Mean | | 5.43 | - | 12.46 | | | |
| 3 | 45 | 6 | - | 10 | - | 18 | 39.96 |
| | | 4 | - | 10 | 4 | 15 | 33.3 |
| | | 6 | - | 9 | 2 | 3 | 6.66 |
| | | 5 | - | 12 | - | 6 | 13.32 |
| | | - | - | 22 | - | 3 | 6.66 |
| | | | | | | | |
| Range | | 0-6 | - | 9-22 | 0-4 | | |
| Mean | | 4.81 | - | 11.00 | 1.46 | | |

Table - 130

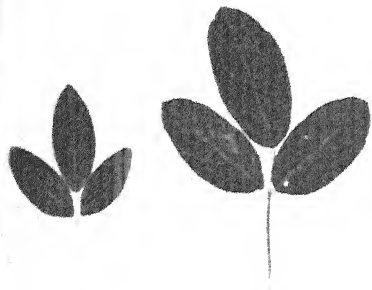
Chiasma frequency at M - I in induced tetraploids of Alysicia cajaniifolia
 (C_0 and C_1)

| Genera- tion | No. of cells studied | No. of quadrivalents with 3xmata | 4xmata | No. of tri- valents | No. of bivalents with 2xmata | 1xmata | No. of univa- lents | No. of Total xmata per cell |
|----------------------------------|----------------------------|--|--------|------------------------|------------------------------------|--------|------------------------|-----------------------------------|
| C ₀ | 45 | 40 | 154 | 6 | 426 | 91 | 30 | 1794 39.8 |
| C ₁ Plant No. 1 | 40 | 24 | 150 | - | 400 | 138 | 96 | 1610 40.25 |
| Plant No. 2 | 39 | 14 | 198 | - | 300 | 186 | - | 1620 41.53 |
| Plant No. 3 | 35 | 16 | 200 | - | 405 | 90 | 66 | 1748 38.84 |

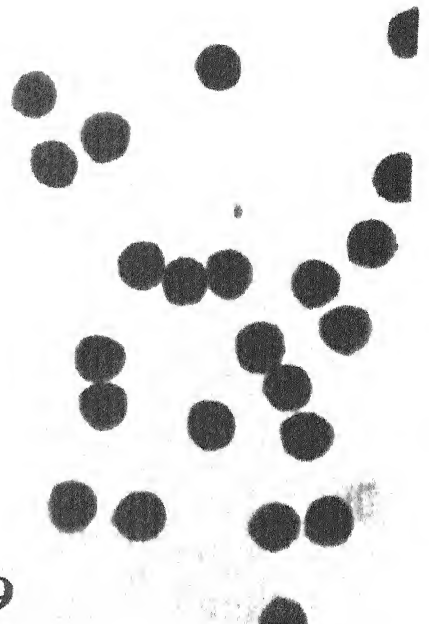
Chromosome distribution at Anaphase - I in induced tetraploids of Atylosia calanifolia
(C₀ and C₁) (% in parentheses)

| Generation | No. of cells studied | Normal separation | Unequal distribution | No. of cells studied | Sporad Stage | Micro-nuclei. | pollen fertility | pollen size | Mean |
|----------------|----------------------|-------------------|----------------------|----------------------|--------------|---------------|------------------|-------------|------------|
| | | | | | Tetrad | | | Range | (μ) |
| C ₀ | 50 | 47 (94.0) | 1 (2.0) | 2 (4.0) | 85 | 75 (88.92) | 3 (3.52) | 6 (7.02) | 42-51 48.2 |
| C ₁ | 65 | 63 (96.39) | - | 2 (3.07) | 90 | 86 (95.46) | 3 (3.33) | 1 (1.11) | 39-51 46.8 |
| Plant No. 1 | 51 | 49 (94.08) | 2 (3.92) | - | 105 | 99 (95.28) | 2 (1.90) | 4 (3.80) | 42-51 49.2 |
| Plant No. 3 | 46 | 46 (100) | - | - | 87 | 87 (100) | - | - | 42-54 51.3 |

PLATE - 20



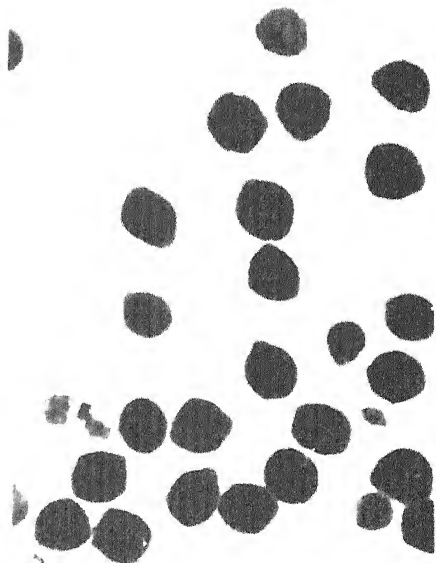
2



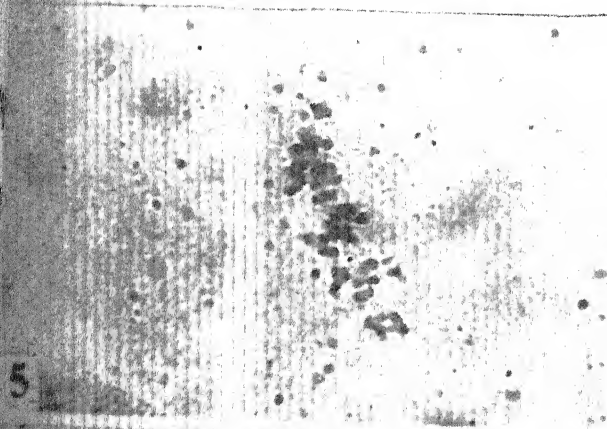
9



4



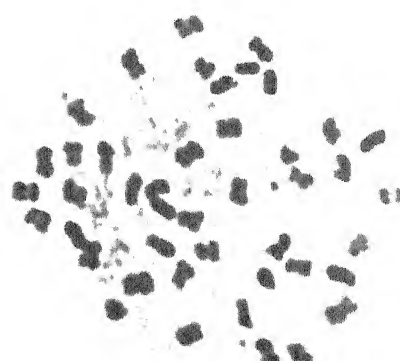
10



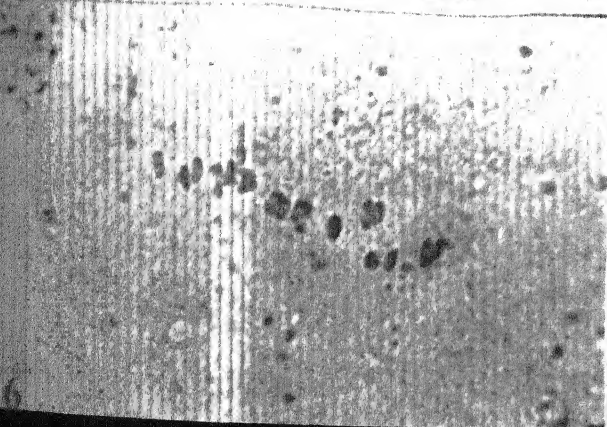
5



7



8



6

Univalents ranged from 0-4 with 1.46 univalents per cell. Maximum number of cells were observed with chromosomal associations of 6 IVs + 10 IIs (39.96%). Chiasma frequency as observed at metaphase-I was 38.84 per cell (Table-130). At anaphase-I, equal separation of chromosomes to the poles was observed in all the cells studied. At sporad stage only tetrad formation was observed. Pollen fertility percentage was 93.6 and fertile pollen size ranged from 42-54 u with 51.3 u mean diameter (Table-131).

Observations on the effects of colchicine in *Atylosia volubilis*.

a) Seed germination:

The effects of colchicine on seed germination at different concentrations and durations of treatments are summarised in (Table-132). Details are as follows:

In the treatment with 0.05% colchicine solution for 4, 6 and 8 hours percentage seed germination was observed as 90.0, 80.0 and 70.0 respectively. However, in the prolonged treatment for 24 hours no seed germination was observed. In the treatment with 0.1% colchicine applied for 4, 6 and 8 hours, seed germination percentage was 80.0, 70.0 and 60.0 respectively. No seed germination was noticed when 0.1% colchicine solution was applied for 24 hours. In the treatment with 0.2% colchicine solution for periods of 2, 4, 6 and 8 hours, seed germination percentage was 80.0, 80.0, 60.0 and 20.0 respectively. The time taken by treated seeds for germination varied from 2-8 days while the untreated seeds germination in 2-4 days.

b) Plant survival:

The effects of colchicine on plant survival was studied in seed and seedling treatment (Table-132). Survival

percentage differed in both the treatments.

In the seed treatment, plant survival varied from 0-10%. The highest survival (10%) was recorded with 0.05% colchicine treated for 4 and 6 hours. While in longer duration treatments (for 8 and 24 hours) plants could not survive. Similarly in the remaining treatments i.e. 0.1% applied for 4, 6, 9 and 24 hours, 0.2% for 2, 4, 6 and 8 hours, plants could not survive. The seed treatment was not successful. The cause of failure appeared to be the drastic effect of colchicine on roots, which failed to produce lateral roots and hence seedlings could not develop properly after respective treatments. The plants which survived after the treatments were also found to be diploid on their cytological examination.

When seedlings were immersed in 0.05% colchicine solution for 4, 6 and 8 hours, percentage seedling survival was 60.0, 40.0 and 40.0 respectively. Those seedlings immersed in 0.1% colchicine solution for 4, 6 and 8 hours, the survival percentage was 12.0, 4.0 and 10.0 respectively. Four per cent seedlings survived after the treatment with 0.2% colchicine for a period of 2 hours. In other treatments for 4, 6 and 8 hours no seedling survival was recorded.

Colchicine treatment of seedlings through absorbent cotton plug soaking method exhibited differential survival of seedlings at different concentrations and durations. In the treatments of 0.05% colchicine for 8 hours a day for one, two and three days, seedling survival percentage was 100, 80.0 and 80.0 respectively. 0.1% colchicine solution when applied for 8 hours a day for one, two and three days survival of seedlings was 93.3%, 86.6% and 83.3% respectively. In the treatments of 0.2% colchicine for 8 hours a day for one, two and three days, the survival

percentage of seedlings were 80.0, 60.0 and 13.3 respectively.

c) Production of polyploid:

Polyploid could not be induced in seed as well as seedling immersion method treatments. Chromosome doubling was successfully induced through the apical bud treatment wherein 0.2% colchicine applied for 8 hours a day for 3 days (Table-132) through absorbent cotton plug soaked with colchicine solution.

Studies on induced tetraploid of *Atylosia volubilis*.

a) Morphology:

Comparative morphological characters of diploid and induced tetraploid *Atylosia volubilis* (C_0 and C_1) are summarised in Table-133. Details observations pertaining to the morphology of diploid and induced tetraploids of *Atylosia volubilis* are as follows:

1. Seedling, branches and plant spread:

Immediately after treatment of the apical buds of the seedlings, the first pair of leaves gradually become darker green in colour and thicker than the untreated ones. The induced tetraploid of *Atylosia volubilis* showed less number of primary as well as secondary branches in comparison to its diploid counterpart. The number of primary and secondary branches in diploid and tetraploid plants were 9 and 12; 3 and 4 respectively. C_0 tetraploid plant showed reduced plant spread (35.0 cm) as compared to diploid (82.0 cm). Stem of induced tetraploid had shorter internodes in comparison to its diploid.

In the C_1 generation, first pair of simple leaves were darker green in colour and thicker than their diploids. In C_1 plants, the number of primary and secondary branches ranged from 6 to 18 and 12 to 26 respectively. Plant spread in these C_1 plants ranged from 55 to 105, the average being 85.0.

2. Days to flowering and maturity:

In contrast to diploids, delay in flowering as well as maturity was recorded in the induced tetraploids. After sowing, the C_0 plant took 175 days for bud initiation and 227 days for 50% flowering, whereas, days for bud initiation and 50% flowering in diploid plants were 150 and 208 respectively. Days taken by bud for full development into flower and from pod initiation to maturity in C_0 plant and the diploid were 21 and 15; 42 and 38 respectively. Days to 50% pod maturity were recorded to be 267 and 273 in tetraploid and diploid respectively.

In C_1 plants, days from sowing to bud initiation ranged from 165 to 181 and days from sowing to 50% flowering ranged from 197 to 219. The days taken by bud for full development into flower and from pod initiation to maturity ranged from 16 to 20 and 38 to 42 respectively. Days to 50% pod maturity ranged from 270 to 290 in these C_1 plants.

3. Leaf:

The leaves of C_0 plants were comparatively thicker and darker green in colour to its diploid counterpart. Marked increase in size of leaves of C_0 plants was observed (Plate-21; Fig. 1). The central leaf let length

and breadth in C_0 plant was 5.1 cm and 4.8 cm as against 4.2 cm and 4.0 cm in diploid. The average petiolar length (3.2 cm) was observed in induced tetraploid while it was 3.5 cm in the diploid. The surface of leaves of diploid as well as tetraploid plants was non-hairy.

In C_1 generation Central leaf let length and breadth of induced tetraploid plants ranged from 5.5 to 6.2 cm and 5.4 to 6.0 cm respectively. Petiolar length in these C_1 plants ranged from 4.6 to 5.2 with 4.9 average petiolar length. The leaves of C_1 plants were also thicker and darker green in colour. In all the C_1 plants, the leaves were non-hairy.

4. Flower:

The C_0 plant produced larger flowers as compared to those of diploid. The size of standard petal of C_0 plant was 3.42 cm^2 as against 2.72 cm^2 in diploid. Similarly the length of style was also 1.8 cm and 1.6 cm in tetraploid and diploid respectively.

In C_1 plants, the size of standard petal ranged from 3.20 to 3.45 cm^2 , the average being 3.38 cm^2 . Increase in stylor length was also observed as it ranged from 1.8 to 1.9 cm, the average being 1.9 cm.

5. Pod setting:

The induced tetraploid plant of A. volubilis showed 4.0% pod setting in C_0 generation. In C_1 , it ranged from 9.6 to 21.0%, the average being 15.0%.

6. Pod:

Tetraploid plant had reduced pod size as it was

1.8 cm² in tetraploid (C₀) and 2.6 cm² in diploid. Pods of C₀ plant showed 0.620 cm thickness while it was 0.504 cm in diploid. Pods of diploid as well as tetraploid plants were non-hairy. Number of chambers per pod and number of seeds per pod in C₀ plant was 2.3 and 0.8 respectively as against 3.0 and 2.3 in diploid.

In C₁ plants, pod sizes ranged from 1.9 to 2.6 cm², the average being 2.2 cm². Thickness of pod ranged from 0.59 cm to 0.70 cm with an average of 0.60 cm. In these plants, the number of chambers per pod ranged from 1-3 and number of seeds per pod 0.9 to 1.6, the average being 1.10 seeds per pod. All the C₁ plants possessed dense-hairy pods.

6. Ovule fertility:

Percentage fertility of ovule was 34.78 in tetraploid as against 66.5 in the diploid. In C₁ plants, it ranged from 26.5 to 51.2%, the average ovule fertility being 40.0%.

7. Seed:

The seeds of C₀ plants were thicker and more bold in comparison to diploid. Average thickness of seed was 0.33 cm in C₀ plant as against 0.298 cm in diploid. In C₁ plants average seed thickness ranged from 0.300 cm to 0.400 cm, the average being 0.33 cm seed thickness.

8. Stomata:

Considerable increase in the size of stomata in tetraploid plants over the diploid (Plate-21; Figs. 2,3) was noticed. The length and breadth of stomata of C₀ plant was 18.0 u and 12 u in diploid. However, the tetraploid

exhibited reduction in number of stomata per unit area (6.0) as compared to diploid (8.0).

In C_1 plants too, reduction in the number of stomata per unit area was observed and the mean value of 6.2 stomata per unit area was recorded. In these plants, the size of stomata ranged from 206 μ to 279 μ , the average being 268 μ .

b) Cytology (C_0).

Mitosis:

Mitotic studies in root tip cells of colchicine treated seeds of A. volubilis revealed different ploidy levels as $4n$ and $8n$ (Plate-24; Figs. 8,9,10) (Table-134) at different concentrations and durations. The lowest concentration (0.025%) colchicine solution used for 6 hours brought about only condensation of chromosomes. While in 0.05% cells exhibited chromosome doubling ($4n = 44$). In the treatment with 0.1% concentration and 6 hours duration, 46.2% cells were observed having 44 chromosomes and in remaining 52.8% cells, 22 chromosomes were observed. In the treatment with 0.2% colchicine for 2 and 4 hours, 44 chromosomes were observed in 9.99% and 66.0% cells respectively and in the remaining 89.9 and 33.0 per cent cells, 22 condensed chromosomes were noticed. When 0.2% colchicine solution was applied for 6 and 8 hours, cells with $4n$ and $8n$ ploidy levels were observed, the percentage of such cells were 97.2 and 2.77; 45.0 and 55.0 respectively. 0.2% colchicine solution when applied for 6 hours, maximum cells (97.2%) showed $4n = 44$ chromosomes. The highest concentration (0.2%) of colchicine used for 8 hours, resulted in an increase in percentage of cells with more than $4n$ ploidy level (Table-134). Octoploidy

level was observed in 55.0% of cells and in remaining 45.0% cells it was tetraploidy.

Meiosis:

Meiotic studies in C_0 plant revealed various chromosomal associations as quadrivalent, trivalent, bivalent and univalent at metaphase-I. It is clear from Table-135 that at metaphase-I, quadrivalents ranged from 3-8 with 5.34 per cell. Bivalents and univalents ranged from 4-18 and 0-4 with 9.34 and 0.69 per cell respectively. The maximum number of 8 quadrivalents were observed in 38.41% cells. While maximum (26.88%) cells were observed with 9 IVs + 6 IIs (Plate-21; Fig. 4,5) chromosomal association at metaphase-I. Maximum number of 4 univalents were observed in 11.53% cell. Chiasma frequency as recorded at metaphase-I was 41.88 per cell (Table-137). At anaphase-I, unequal distribution of chromosomes to the poles was observed in 6.6% cells and in remaining 93.24% cells equal separation of chromosomes to the poles was observed (Table-138). At sporad stage, tetrads, polyads (Plate-21; Fig. 8) and micronuclei were noticed in 97.09%, 1.33% and 2.56% cells respectively. Pollen fertility was 75.8%. A significant increase in fertile pollen size (Plate-21; Figs. 9, 10) was observed in induced tetraploid as it ranged from 21-51 μ with 46.0 μ mean diameter, while in diploid, it ranged from 30-36 μ .

Cytology (C_1).

a) Mitosis:

Mitotic study of C_1 plant revealed $4n = 44$ chromosomes (Plate-21; Fig. 11) at somatic metaphase of root tip cells.

b) Meiosis:

Meiotic studies were carried out in 3 tetraploid plants separately and the observations are as follows:

Plant No. 1:

Data on chromosomal associations at M-I revealed pollen grain mother cells with varying number of quadrivalents, and bivalents (Table-136). In this plant, quadrivalents ranged from 0-7 with 2.8 per cell and bivalents ranged from 8-22 with 16.2 per cell. Univalents ranged from 0-4 with 0.26 per cell. Maximum number of 7 quadrivalents were observed in 13.33% cells. And maximum percentage of cells (33.3) was observed with 22 bivalents at metaphase-I. Chromosomal association of 3 IVs + 16 IIs (Plate-21; Fig.5) was observed in 13.3% cells. Chiasma frequency as observed at metaphase-I was 40.60 per cell (Table -137). At anaphase-I equal separation of chromosomes was observed in all the cells studied (Table-138). At sporad stage, micronuclei were observed in 3.75% cells and in remaining 96.25% cells tetrad formation was observed. Pollen fertility was 77.1% and fertile pollen size ranged from 42-48 μ with 45.70 μ mean diameter.

Plant No. 2:

In this plant quadrivalents, bivalents and univalents were observed at metaphase-I (Table-136). The quadrivalents ranged from 3-7 with 5.46 per cell. Maximum number of 7 quadrivalents were observed in 28.57% cells and minimum 3 quadrivalents in 14.28% cells. At metaphase-I, bivalents ranged from 8-16 with 10.64 per cell and univalents ranged from 0-4 with 0.5 per cell. Chiasma frequency as observed at metaphase-I, was 39.39 per cell

Table - 132

Seed germination and plant survival in *Alysicarpus* .
No. of seeds treated in each case = 10. (% in parentheses)

| Seed treatment | | | Seedling treatment (Immersion Method) | | | Seedling treatment (drop through cotton plug method) | | | | | | |
|---------------------------|-------------------------|--------------------------|--|---------------------------|-------------------------|---|---------------------------------|---------------------------|--------------------------------|-------------------------------------|---|---|
| Concen- tration (%) | Dura- tion (hrs.) | Seeds germi- nated | Seed- lings survi- ved | Concen- tration (%) | Dura- tion (hrs.) | No. of seed- lings treated | Seed- lings survi- ved | Concen- tration (%) | Dura- tion hrs./ days | No. of seed- lings treated | Seed- Tetra- lings ploid survi- plants ved | |
| 0.05 | 4 | 9 (90.0) | 1 (10.0) | 0.05 | 4 | 10 | 6 (60.0) | 0.05 | A ₁ | 10 | 10 (100) | 0 |
| 0.05 | 6 | 8 (80.0) | 1 (10.0) | 0.05 | 6 | 10 | 4 (40.0) | 0.05 | A ₂ | 10 | 8 (80.0) | 0 |
| 0.05 | 8 | 7 (70.0) | 0 | 0.05 | 8 | 10 | 4 (40.0) | 0.05 | A ₃ | 10 | 8 (80.0) | 0 |
| 0.05 | 24 | 0 | 0 | 0.05 | 24 | 10 | 0 | 0.05 | A ₁ | 10 | 0 | 0 |
| 0.01 | 4 | 8 (80.0) | 0 | 0.1 | 4 | 50 | 6 (12.0) | 0.1 | A ₁ | 30 | 28 (93.3) | 0 |
| 0.1 | 6 | 7 (70.0) | 0 | 0.1 | 6 | 50 | 2 (4.0) | 0.1 | A ₂ | 30 | 26 (86.6) | 0 |
| 0.1 | 8 | 6 (60.0) | 0 | 0.1 | 8 | 50 | 1 (2.0) | 0.1 | A ₃ | 30 | 25 (83.33) | 0 |
| 0.1 | 24 | 0 | 0 | 0.1 | 24 | 50 | 0 | 0.1 | A ₁ | 30 | 24 (80.0) | 0 |
| 0.2 | 2 | 8 (80.0) | 0 | 0.2 | 2 | 25 | 2 (8.0) | 0.2 | A ₁ | 30 | 18 (60.0) | 0 |
| 0.2 | 4 | 8 (80.0) | 0 | 0.2 | 4 | 25 | 0 | 0.2 | A ₂ | 30 | 18 (60.0) | 0 |
| 0.2 | 6 | 6 (60.0) | 0 | 0.2 | 6 | 25 | 0 | 0.2 | A ₃ | 30 | 4 (13.3) | 1 |
| 0.2 | 8 | 2 (20.0) | 0 | 0.2 | 8 | 25 | 0 | 0.2 | A ₃ | 30 | 1 (3.33) | 0 |

A₁ = 8 hrs. - One day; A₂ = 8 hrs. - 2 days; A₃ = 8 hrs. - 3 days.

Table - 133

Comparative morphology of diploid and induced tetraploid of Atylosia volubilis

| Characters | <u>A. volubilis</u> | <u>A. volubilis</u> | <u>A. volubilis</u> |
|---|---------------------|----------------------|----------------------|
| | 2x | 4x (C ₀) | 4x (C ₁) |
| No. of primary branches | 9 | 3 | 11 |
| No. of secondary branches | 12 | 4 | 18 |
| Central leaflet: surface | Non-hairy | Non-hairy | Non-hairy |
| (L x B) cm. | 4.2 x 4.0 | 5.1 x 4.8 | 5.8 x 5.6 |
| length of petiole (cm) | 3.5 | 3.2 | 4.9 |
| Spread of plant (cm) | 82.0 | 35.0 | 85.0 |
| Days from sowing to bud initiation | 150 | 175 | 170 |
| Days from sowing to flowering | 20.8 | 227 | 212 |
| Days between bud to flower | 15 | 21 | 18 |
| Days between pod initiation to maturity | 38 | 42 | 40 |
| Size of the standard petal (L x B) cm. | 1.7 x 1.6 | 1.9 x 1.8 | 1.88 x 1.8 |
| Length of style (cm) | 1.60 | 1.80 | 1.85 |
| Pod (L x B) cm | 2.6 x 1.0 | 1.8 x 1.0 | 2.2 x 1.0 |
| Thickness of pod (cm.) | 0.504 | 0.620 | 0.600 |
| Hairs on mature pod | Absent | Absent | Absent |

(Contd... 2)

| 1 | 2 | 3 | 4 |
|-------------------------|---------|---------|-------------|
| No. of chambers per pod | 3.0 | 2.3 | 2.6 |
| No. of seeds per pod | 2.3 | 0.8 | 1.10 |
| Thickness of seed (cm) | 0.208 | 0.300 | 0.330 |
| Days to maturity | 273 | 287 | 276 |
| Pod set (%) | 52.00 | 4.0 | 15.0 |
| Ovule fertility (%) | 66.5 | 34.78 | 40.00 |
| Stomata: frequency | 8.0 | 6.0 | 6.2 |
| (L x B) μ | 15 x 12 | 18 x 15 | 17.8 x 15.1 |
| | | | 296 |

Table - 134
Effects of colchicine on somatic chromosomes of Atylosia volubilis
(% in parentheses)

| Concentration (%) | Duration (hours) | No. of cells studied | PLOIDY LEVEL AT METAPHASE | | | |
|-------------------|------------------|----------------------|---------------------------|--------------|--------------|-----|
| | | | 2n | 4n | 8n | 16n |
| 0.025 | 6 | 25 | 25 (100) | - | - | - |
| 0.05 | 6 | 25 | 24 (96.0) | 1 (4.0) | - | - |
| 0.1 | 6 | 30 | 16 (52.8) | 14 (46.2) | - | - |
| 0.2 | 2 | 30 | 27 (89.99) | 3 (9.99) | - | - |
| " | 4 | 30 | 10 (33.0) | 20 (66.0) | - | - |
| " | 6 | 36 | - | 35 (97.2) | 1 (2.77) | - |
| " | 8 | 40 | - | 18 (45.0) | 22 (55.0) | - |

Table - 135

Chromosomal associations at Metaphase - I in Atylosia
volubilis (C₀)

| No. of cells studied | Chromosomal associations at M - I | | | | Fre- quency | Per cent |
|----------------------------|--------------------------------------|-----|------|------|----------------|----------|
| | IV | III | II | I | | |
| 52 | 8 | - | 5 | 2 | 6 | 11.53 |
| | 8 | - | 6 | - | 14 | 26.88 |
| | 7 | - | 8 | - | 12 | 23.07 |
| | 6 | - | 10 | - | 3 | 5.76 |
| | 5 | - | 10 | 4 | 6 | 11.53 |
| | 4 | - | 18 | - | 5 | 9.60 |
| | 3 | - | 16 | - | 6 | 11.53 |
| Range | 3-8 | - | 4-18 | 0-4 | | |
| Mean | 6.34 | - | 9.34 | 0.69 | | |

Table - 136

Chromosome associations at Metaphase - I in induced tetraploid of Atylosia volubilis (C₁)

| Plant No. | No. of cells studied | Chromosomal associations at M-I | | | | Frequency | Per cent |
|-----------|----------------------|---------------------------------|-----|-------|------|-----------|----------|
| | | IV | III | II | I | | |
| 1 | 30 | 7 | - | 8 | - | 4 | 13.33 |
| | | 6 | - | 10 | - | 6 | 19.98 |
| | | 3 | - | 16 | - | 4 | 13.33 |
| | | 2 | - | 18 | - | 4 | 13.33 |
| | | - | - | 20 | 4 | 2 | 6.66 |
| | | - | - | 22 | - | 10 | 33.3 |
| Range | | 0-7 | - | 8-22 | 0-4 | | |
| Mean | | 2.8 | - | 16.2 | 0.26 | | |
| 2 | 28 | 7 | - | 7 | 2 | 8 | 28.57 |
| | | 6 | - | 10 | - | 5 | 17.85 |
| | | 5 | - | 12 | - | 6 | 21.42 |
| | | 5 | - | 10 | 2 | 3 | 10.71 |
| | | 5 | - | 9 | 4 | 2 | 7.14 |
| | | 3 | - | 16 | - | 4 | 14.28 |
| Range | | 3-7 | - | 8-16 | 0-4 | | |
| Mean | | 5.46 | - | 10.64 | 0.5 | | |
| 3 | 24 | 6 | - | 10 | - | 5 | 20.83 |
| | | 5 | - | 12 | - | 8 | 33.28 |
| | | 3 | - | 16 | - | 4 | 16.64 |
| | | 3 | - | 13 | 6 | 7 | 29.12 |
| Range | | 3-6 | - | 10-16 | 0-6 | | |
| Mean | | 4.29 | - | 12.54 | 1.17 | | |

Table - 137
Chiasma frequency at Metaphase - I in induced tetraploids of Atylosia volubilis.

| plants and genera- tion | No. of cells studied | No. of Quadri- valent | | Pivalents with | | No. of univa- lents | Total xmata | xmata per cell |
|----------------------------------|----------------------------|--------------------------|--------|----------------|------|---------------------------|----------------|-------------------|
| | | 3xmata with | 4xmata | 2xmata | 1xma | | | |
| C ₀ | 52 | 28 | 302 | 400 | 86 | 36 | 2178 | 41.88 |
| C ₁ Plant No. 1 | 30 | 4 | 80 | 400 | 86 | 8 | 1218 | 40.60 |
| Plant No. 2 | 28 | 10 | 143 | 203 | 95 | 14 | 1103 | 39.39 |
| Plant No. 3 | 24 | 3 | 100 | 251 | 50 | 42 | 961 | 40.04 |

Table - 138

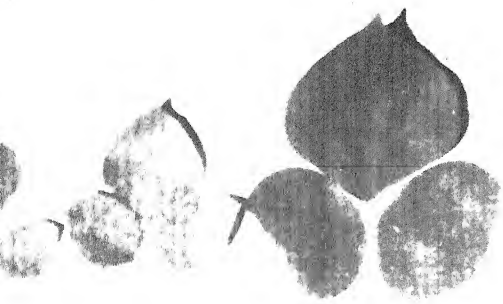
Chromosome distribution at anaphase - I in induced tetraploids of Atylosia volubilis
(figures in parentheses are %)

| Generation | No. of cells studied | Normal separation | unequal distribution | Laggards | Sporad Stage | | | Pollen fertility % | Pterile size Range | pollen Mean (n) |
|-------------------------------|----------------------|-------------------|----------------------|-------------|----------------------|-------------|--------------------|--------------------|--------------------|-----------------|
| | | | | | No. of cells studied | Tetrad | polyad nuclei. (n) | | | |
| C ₀ | 45 | 42 (93.24) | 3 (6.66) | - | 75 (97.09) | 1 (1.33) | 2 (2.66) | 75.8 | 21-51 | 46.0 |
| C ₁ Plant No. 1 | 38 | 38 (100) | - | - | 75 (96.25) | - | 3 (3.75) | 77.1 | 42-48 | 45.7 |
| Plant No. 2 | 41 | 36 (87.66) | 2 (4.87) | 3 (8.49) | 85 (93.6) | - | 5 (5.88) | 80.2 | 42-48 | 44.6 |
| Plant No. 3 | 30 | 29 (96.66) | - | 1 (3.33) | 75 (98.68) | - | 1 (1.31) | 82.8 | 42-48 | 45.4 |

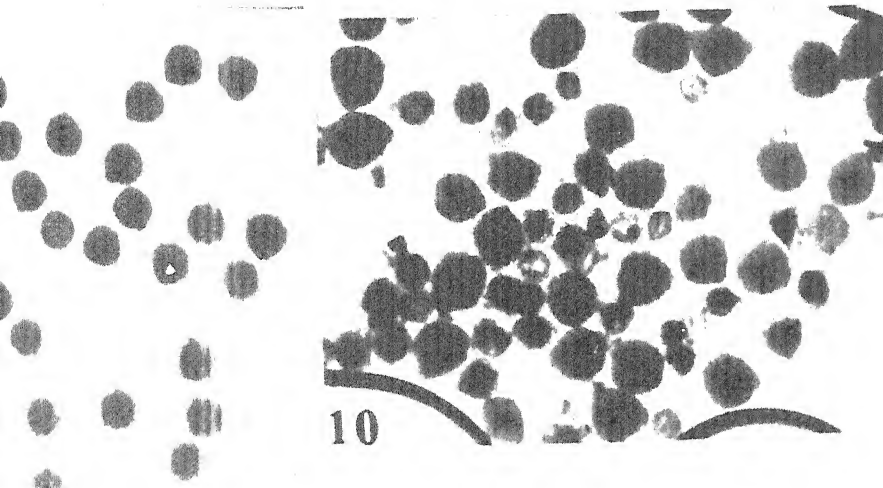
PLATE - 21 (Induced tetraploid of A. volubilis)

- Fig. 1. Leaves of diploid and tetraploid
(2 left one - diploid, one right - tetraploid)
- Fig. 2. Stomata of diploid (C_0) (X 600)
- Fig. 3. Stomata of tetraploid (C_0) (X 600)
- Fig. 4. 8 IV's + 6 II's at Metaphase-I (C_0) (X 1500)
- Fig. 5. 8 IV's + 5 II's + 2 I's at Metaphase-I (C_0)
(X 1500)
- Fig. 6. 3 IV's + 16 II's at Metaphase-I (C_1)
No. 1 (X 1500)
- Fig. 7. 6 IV's + 10 II's (C_1) No. 3 (X 1500)
- Fig. 8. Hexad with tetrad (C_0) (X 400)
- Fig. 9. Pollen grains of diploid (X 600)
- Fig. 10. Pollen grains of tetraploid (X 600)
- Fig. 11. 44 Somatic chromosomes at Metaphase-I
(C_1) (X 1500)

PLATE - 21



5



8



(Table-137). At anaphase-I, unequal distribution of chromosomes and laggards were observed in 4.87% and 8.49% cells respectively. In remaining 87.66% cells normal separation of chromosomes to the poles was noticed. At sporad stage, formation of micronuclei was observed in 5.88% cells and in remaining 93.6% cells tetrads were recorded (Table-138). Pollen fertility percentage was 80.2 and fertile pollen size ranged from 42-48 μ with 44.6 μ mean diameter (Table-138).

Plant No. 3:

At metaphase-I, quadrivalents ranged from 3-6 with 4.29 per cell. Bivalents and univalents ranged from 10-15 and 0-6 with 12.54 and 1.17 per cell respectively. At metaphase-I maximum number of 6 quadrivalents (Plate-21; Fig. 7) and 6 univalents were observed in 20.83 and 29.12% cells respectively. Chiasma frequency at metaphase-I was 40.04 per cell (Table-137). At anaphase-I, laggards were observed in 3.33% cells and in remaining 96.66% cells equal separation of chromosomes to the poles was observed (Table-138). At sporad stage, micronuclei were seen in 1.31% cells and remaining 98.68% cells met with regular tetrad formation.

Pollen fertility was 82.8% and fertile pollen size ranged from 42 to 48 μ with 45.4 μ mean diameter (Table-138).

Observations on the effects of colchicine in *Atylosia scarabaeoides*.

a) Seed germination:

The effects of colchicine on seed germination at different concentrations and durations of treatments in *A. scarabaeoides* (Table-139) are as follows:

In the treatment with 0.05% colchicine solution for 4, 6, 8 and 24 hours, percentage seed germination was observed as 100, 90.0, 90.0 and 60.0 respectively. In the treatment with 0.1% colchicine applied for 4, 6, 8 and 24 hours, observed seed germination percentage was 80.0, 60.0, 60.0 and 50.0 respectively. In the treatment with 0.2% colchicine solution for periods of 2, 4, 6 and 8 hours, seed germination percentage was 90.0, 80.0, 70.0 and 40.0 respectively. The time taken by treated seeds for germination varied from 2-8 days while the untreated seeds germinated in 1-4 days.

b) Plant survival:

The effects of colchicine on plant survival was studied in the experiments on seed and seedling treatments (Table-139). Plant survival percentage differed in both the treatments.

In the seed treatments, plant survival varied from 0-50%. The highest (50.0%) survival was recorded in the treatment of 0.05% colchicine for 4 hours duration. In the treatments with 0.05% colchicine used for 6 and 8 hours 30.0% and 10.0% plants survived after the respective treatments. While in longer duration treatments (24 hours) no seedling could emerge. In the treatments with 0.1% colchicine applied for 4, 6, 8 and 24 hours, seedling could not emerge. Similar results were seen in the treatments with 0.2% colchicine used for 2, 4, 6 and 8 hours.

When seedlings were immersed in 0.05% aqueous colchicine solution for 4, 6 and 8 hours, percentage survival was 80.0, 80.0 and 70.0 respectively. Those seedlings immersed in 0.1% colchicine solution for 4, 6, and 8 hours, the survival percentage was 50.0, 40.0 and

20.0 respectively. After the treatments with 0.2% colchicine for 2, 4, 6 and 8 hours, 10.0, 5.0 and 5.0%, plants survived respectively.

Colchicine treatment of seedlings through absorbent cotton plug soaking method exhibited differential survival of seedlings at different concentrations and durations. In the treatment of 0.05% colchicine for 8 hours a day for one, two and three days, seedling survival percentage was 100.0, 100.0 and 80.0 respectively. 0.1% colchicine solution when applied for 8 hours a day for one, two and three days, 83.33, 66.66 and 59.94 per cent seedlings survived respectively. In the treatments of 0.2% colchicine for 8 hours a day for one, two and three days, the survival percentage of seedlings were 83.33, 20.0 and 16.55 respectively.

c) Production of polyploids:

Polyploidy could not induced through seed treatment. The seedlings treated with 0.2% colchicine for 8 hours resulted in chimera plant. Chromosome doubling was successfully induced through the apical bud treatment wherein 0.2% colchicine solution applied for 8 hours a day for three days was found to be effective.

Studies on induced tetraploid of *Atylosia scarabaeoides*.

a) Morphology:

Comparative morphological characters of diploid and induced tetraploid of *Atylosia scarabaeoides* are summarised in Table-140. Details observations pertaining to the morphology of diploid and induced tetraploids of *Atylosia scarabaeoides* are as follows:

1. Seedling; branches and plant spread:

After the treatment of apical buds of seedlings, the first pair of leaves gradually became darker green in colour and thicker than the untreated ones. The induced tetraploid of Atylosia scarabaeoides showed less number of primary as well as secondary branches in comparison to its diploid counterpart. The number of primary and secondary branches in diploid and tetraploid plants were 8 and 11; 3 and 5 respectively. C_0 plant showed reduced plant spread (22.0 cm) as compared to diploid (40.0 cm). Stem of induced tetraploid had shorter internodes in comparison to its diploid.

In the C_1 generation, first pair of simple leaves were darker green in colour and thicker than their diploids. In C_1 plants, the number of primary and secondary branches ranged from 6 to 12 and 10 to 21 respectively. Plant spread in these plants ranged from 38.0 to 63.0 cm, the average being 51.5 cm.

2. Days to 50% flowering and maturity:

In contrast to diploids, delay in flowering as well as maturity was recorded in the induced tetraploids.

After sowing, the C_0 plant taken 113 days for bud initiation and 128 days for 50% flowering. Whereas, days for bud initiation and 50% flowering in diploid plants were 88 and 99 respectively. The days taken by bud for full development into flowers were 9 and 13 in diploid and induced tetraploid respectively. The time from pod initiation to maturity was observed to be 40 and 36 in tetraploid and diploid respectively. Days to 50% pod

maturity were observed to be 177 and 153 in C_0 plant and the diploid respectively.

In C_1 plants, days from sowing to bud initiation ranged from 95 to 107 and the days from sowing to 50% flowering 118 to 128. On an average, the days taken by buds for full development into flower ranged from 11 to 14 and the days from pod initiation to maturity 35 to 42 in these C_1 plants. Days to 50% pod maturity ranged from 165 to 178.

3. Leaf:

The leaves of C_0 plants were comparatively thicker and darker green in colour to its diploid counterpart. Considerable increase in size of leaves of C_0 plant was observed (Plate-22; Fig. 1). The leaf length and breadth of C_0 plant was 3.15 cm and 2.61 cm respectively as against 2.74 cm length and 1.40 cm breadth in diploid. The average petiolar length was 1.6 cm as observed in tetraploid while it was 1.5 cm in the diploid. The surface of leaves of induced tetraploids was more hairy as compared to diploid.

In C_1 generation, leaf length and breadth of tetraploids ranged from 3.0 to 4.2 cm and 2.2 to 3.1 cm respectively. Petiolar length in these C_1 plants ranged from 1.5 to 1.9 cm, the average being 1.71 cm. Dense hairy leaves were the characteristic feature of all the C_1 plants studied.

4) Flower:

The C_0 plants produced larger flowers as compared to those of diploid. The size of standard petal of C_0 plant was 0.73 cm^2 as against 0.355 cm^2 in diploids. Similarly

the length of style was also increased over the diploid (0.90 cm in tetraploids; 0.70 cm in diploids).

In C_1 plants, the size of standard petal ranged from 0.70 to 0.93 cm², the average being 0.825 cm². Stylar length ranged from 0.90 to 1.10 cm, the average being 1.0 cm.

5) Pods

The induced tetraploid plant of Atylosia scarabaeoides showed reduced pod setting (12.0%) in comparison to 64.0% in diploid. In C_1 plants, it ranged from 21.0 to 32.0 per cent, the average being 26.0 per cent.

Tetraploid plant also showed reduced pod size (Plate-22; Fig. 2). It was 1.24 cm² in the case of C_0 plant and 1.65 cm² in the diploids. Pods of tetraploid plant were more hairy as compared to diploids. On an average number of chambers per pod were 2.6 and 3.40 in tetraploid and diploid respectively. The number of seeds per pod was 1.40 and 3.30 in tetraploid and diploid respectively. Tetraploid possessed more thick pods in comparison to the pods of diploids (Table-140).

In C_1 plants, pod size ranged from 1.26 to 1.82 cm², the average being 1.42 cm². Thickness of pods ranged from 0.300 cm to 0.45 cm, the average being 0.35 cm. In these plants, the number of chambers per pod ranged from 2 to 5, the average being 2.8 and the number of seeds per pod ranged from 1.0 to 5.0, the average being 1.9 seeds per pod. All the C_1 plants possessed hairy pods.

6. Ovule fertility:

Percentage fertility of ovule was 26.92 in tetraploid (C_0) and 90.0 in the diploid. In C_1 plants it ranged from 35.2 to 61.0 per cent, the average being 41.50 per cent.

7. Seeds:

The seeds of C_0 plants were thicker and more bold in comparison to the seeds of diploid plant. Average seed thickness was 0.30 cm in C_0 plants and 0.20 cm in diploids. In C_1 plants seed thickness ranged from 0.25 cm to 0.32 cm, the average being 0.26 cm.

8. Stomata:

Considerable increase was noticed in the size of stomata in tetraploid plants over the diploid (Plate-22; Fig. 3,4). The length and breadth of stomata of C_0 plants was 18.0 μ and 15.0 μ respectively. While it were 12.0 μ and 9.0 μ in diploids. More so, the tetraploid exhibited reduction in number of stomata per unit area (6.0) as compared to diploids (9.0). Reduction in the number of stomata per unit area with the mean value of 5.8 stomata/unit area was registered in the C_1 plants. In these plants the stomata size ranged from 224 μ to 275 μ , the average being 254 μ .

b) Cytology

Mitosis: (C_0)

Mitotic studies in root tip cells of colchicine treated seeds of A. scarabaeoides revealed different ploidy levels as 4x, 8x and 16x (Plate-24; Figs. 4,6) (Table-141) at different concentrations and durations. The lowest

concentration (0.025%) used for 6 hours brought about only condensation of chromosomes. While in 0.05% concentration and 6 hours duration of treatment 3.33% of cells exhibited chromosome doubling ($2n = 4x = 44$). In the treatment with 0.1% concentration and 6 hours duration, 16.50% cells showed presence of 44 chromosomes and in remaining 82.5% of cells, 22 chromosomes were observed. In the treatment with 0.2% colchicine solution for 2 hours, 44 and 22 chromosomes were observed in 20.0% and 80.0% cells respectively. When 0.2% colchicine was applied for 6 hours duration, cells with $4x$, $8x$ and $16x$ ploidy levels were observed, the percentage of such cells were 80.0, 18.0 and 2.0 respectively. The highest concentration of colchicine (0.2%) when used for 6 hours, resulted in the production of higher ploidy as $8x$ and $16x$. Such cells were 47.0 and 11.6 per cent respectively (Table-141).

Meiosis: (C_0).

Meiotic studies in C_0 plants revealed various chromosomal associations as hexavalent, pentavalent, quadrivalent, trivalent, bivalent and univalent (Plate-22; Fig. 5) at metaphase-I. It is clear from Table-142, that at metaphase-I formation of hexavalent and pentavalent ranged from 0-1 and 0-1 with 0.30 and 0.016 per cell respectively. Quadrivalents and trivalents ranged from 0-11 and 0-2 with an average of 4.00 and 0.10 per cell respectively. Maximum number of 11 IVs were observed in 4.8 per cent of cells and 10 IVs + 2 IIs (Plate-22; Fig. 6) were observed in 8.0 per cent cells. At metaphase-I, bivalents and univalents ranged from 0-22 and 0-44 with an average of 10.55 and 3.25 per cell respectively. Maximum number of 22 bivalents (Plate-22; Fig. 7) and 44 univalents (Plate-22; Fig. 8) were observed in 6.4 and 4.8 per cent cells respectively. Chiasma

frequency recorded at metaphase-I was 37.0 per cell (Table-144). At anaphase-I, laggards were observed in 2.22% cells and in remaining cells (97.88%), equal separation of chromosomes to the poles was observed (Table-145). At sporad stage, tetrads and micronuclei were observed in 97.77 and 2.22 per cent cells respectively. Pollen fertility was 72.11 and fertile pollen size ranged from 33 to 48 μ with 36.0 μ mean diameter. Hence a significant increase in pollen size was observed (Plate-22; Figs. 9, 10). In diploids, fertile pollen size ranged from 30-38 μ .

Cytology: (C_1).

a) Mitosis:

Mitotic study of C_1 plants revealed $4n = 44$ chromosomes (Plate-22; Fig. 13) at metaphase of root tip cells.

b) Meiosis:

Meiotic studies were carried out in 3 (C_1) tetraploid plants and the observations are as follows.

Plant No. 1:

Studies on chromosomal associations at metaphase-I revealed PMCs with varying number of quadrivalents, bivalents and univalents (Table-143). Quadrivalents ranged from 0-8 with 4.75 per cell and the maximum number of 8 quadrivalents observed in 33.33 per cent cells. At metaphase-I, bivalents and univalents ranged from 6-22 and 0-44 with 11.41 and 2.25 per cell respectively. Maximum number of 22 bivalents and 44 univalents were recorded in 12.48 and 4.16 per cent

of cells respectively. Chiasma frequency at metaphase-I was 38.41 per cell (Table-144). At anaphase-I, laggards were observed in 6.97 per cent cells and in remaining 92.8 per cent cells, equal separation of chromosomes to the poles was observed (Table-145). At sporad stage, regular tetrad formation was observed in 96.6 per cent cells, except in 3.15 per cent cells where formation of micronuclei was observed (Table-145). Pollen fertility was 89.2 per cent and fertile pollen size ranged from 36 to 45 μ with 37.8 μ mean diameter.

Plant No. 2:

In this plant, quadrivalents, bivalents and univalents were observed at metaphase-I (Table-143). Quadrivalents ranged from 0-8 with 5.57 per cell. Bivalents and univalents ranged from 4-22 and 0-4 with 10.01 and 0.71 per cell respectively. Maximum number of 8 quadrivalents were noticed in 33.87 per cent cells. Maximum number of 22 bivalents and 4 univalents were observed in 15.25 and 8.47 per cent cells respectively (Table-143). Chiasma frequency at metaphase-I was 38.42 per cell (Table-144). At anaphase-I, unequal distribution of chromosomes was observed in 3.27 per cent of cells and in remaining 96.72 per cent cells equal separation of chromosomes to the poles was noticed (Table-145). At the sporad stage, formation of micronuclei was recorded in 2.15 per cent cells and in remaining cell (97.65%), regular tetrad formation was recorded. Pollen fertility was 91.5 per cent and fertile pollen size ranged from 36 to 45 μ with 37.5 μ mean diameter.

Plant No. 3:

In this plant, at metaphase-I, formation of quadrivalents ranged from 0-8 with 5.47 per cell. Maximum

201-1014

Effects of cadhaline on seed germination and plant survival in Atylosia scarabaeoides, (%) in parentheses) No. of seeds treated in each case = 10.

[illegible]

λ_1 = 6 hrs. - one day; λ_2 = 8 hrs. - 2 days; λ_3 = 8 hrs. - 3 days.

Table - 140

Comparative morphology of diploid and induced tetraploid of Atylosia scarabaeoides

| Characters | A. scarab. 2x | A. scarab. 4x (C ₀) | A. scarab. 4 x (C ₁) |
|---|-------------------|------------------------------------|-------------------------------------|
| No. of primary branches | 8 | 3 | 7.6 |
| No. of secondary branches | 11 | 5 | 16.5 |
| Central leaflet: surface | Hairy + | Hairy ++ | Hairy ++ |
| (L x B) cm. | 2.74 x 1.46 | 3.15 x 2.61 | 3.59 x 2.80 |
| length of petiole (cm.) | 1.5 | 1.6 | 1.71 |
| Spread of plant (cm) | 40.0 | 22.0 | 51.5 |
| Days from sowing to bud initiation | 88 | 113 | 98 |
| Days from sowing to flowering | 99 | 128 | 121 |
| Days between bud to flower | 9 | 13 | 12 |
| Days between pod initiation to maturity | 36 | 40 | 38 |
| Size of the standard petal (L x B) cm. | 0.71 x 0.50 | 1.0 x 0.73 | 1.1 x 0.75 |
| Length of style (cm.) | 0.70 | 0.90 | 1.00 |
| pod (L x B) cm. | 2.3 x 0.72 | 1.75 x 0.71 | 2.0 x 0.70 |
| Hairs on mature pod | Present + | Present ++ | Present ++ |
| Thickness of pod (cm.) | 0.31 | 0.46 | 0.35 |
| No. of chambers per pod | 3.40 | 2.6 | 2.8 |
| No. of seeds per pod | 3.30 | 1.40 | 1.9 |
| Thickness of seed (cm.) | 0.20 | 0.30 | 0.260 |
| pod set (%) | 64.0 | 12.0 | 26.0 |
| Ovule fertility (%) | 90.0 | 26.92 | 41.50 |
| Days to maturity | 153 | 177 | 170 |
| Stomata: frequency (L x B) μ | 9.0 12.0 x 9.0 | 6.0 18.0 x 15.0 | 5.8 17.2 x 14.8 |

32
14
23

Table - 141

Effects of colchicine on somatic chromosomes of Atylosia scarabaeoides

| Concentration % | Duration (hours) | No. of cells studied | PLOIDY LEVEL AT METAPHASE | | | |
|--------------------|---------------------|-------------------------|---------------------------|--------------|--------------|-------------|
| | | | 2n | 4n | 8n | 16n |
| 0.025 | 6 | 25 | 25 (100) | - | - | - |
| 0.05 | 6 | 30 | 30 (92.67) | 1 (3.33) | - | - |
| 0.1 | 6 | 30 | 25 (82.5) | 5 (16.50) | - | - |
| 0.2 | 2 | 25 | 21 (80.0) | 5 (20.0) | - | - |
| " | 4 | 25 | 18 (72.0) | 6 (24.0) | 1 (4.0) | - |
| " | 6 | 50 | - | 40 (80.0) | 9 (18.0) | 1 (2.0) |
| " | 8 | 34 | - | 14 (41.1) | 16 (47.0) | 4 (11.6) |

(% in parentheses)

Table - 142

Chromosome associations at Metaphase - I in induced
tetraploid of *Atylosia scarabaeoides* (C₀)

(No. of PMC's studied = 60)

| Chromosomal associations at M-I | | | | | | Frequency Per cent | |
|--------------------------------------|---|----|-----|----|----|--------------------|-----|
| VI | V | IV | III | II | I | | |
| 1 | 1 | 7 | - | 2 | 1 | 1 | 1.6 |
| 1 | - | 6 | - | 7 | - | 2 | 3.2 |
| - | - | 11 | - | - | - | 3 | 4.8 |
| - | - | 10 | - | - | 4 | 2 | 3.2 |
| - | - | 10 | - | 2 | - | 5 | 8.0 |
| - | - | 9 | - | 4 | - | 3 | 4.8 |
| - | - | 8 | - | 6 | - | 3 | 4.8 |
| - | - | 7 | - | 8 | - | 2 | 3.2 |
| - | - | 7 | - | 7 | 2 | 1 | 1.6 |
| - | - | 7 | - | 6 | 4 | 2 | 3.2 |
| - | - | 6 | - | 10 | - | 3 | 4.8 |
| - | - | 5 | - | 12 | - | 2 | 3.2 |
| - | - | 4 | - | 14 | - | 4 | 6.4 |
| - | - | 4 | - | 12 | 4 | 2 | 3.2 |
| - | - | 3 | - | 15 | 2 | 2 | 3.2 |
| - | - | 3 | - | 16 | - | 3 | 4.8 |
| - | - | 2 | 2 | 14 | 2 | 3 | 4.8 |
| - | - | 1 | - | 18 | 4 | 3 | 4.8 |
| - | - | 1 | - | 20 | - | 4 | 6.4 |
| - | - | - | - | 22 | - | 4 | 6.4 |
| - | - | - | - | 21 | 2 | 3 | 4.8 |
| - | - | - | - | - | 44 | 3 | 4.8 |
| Range 0-1 0-1 0-11 0-2 0-22 0-44 | | | | | | | |
| Mean 0.30 0.016 4.80 0.10 10.55 3.25 | | | | | | | |

Table - 143

Chromosome associations at Metaphase - I in induced
tetraploid of Atylosia scarabaeoides (C₁)

| Plant No. | No. of cells studied | Chromosome associations at M - I | | | | Frequ- ency | Per cent |
|--------------|----------------------------|-------------------------------------|-----|-------|-------|----------------|----------|
| | | IV | III | II | I | | |
| 1 | 24 | 8 | - | 6 | - | 8 | 33.33 |
| | | 6 | - | 10 | - | 4 | 16.66 |
| | | 5 | - | 12 | - | 4 | 16.66 |
| | | 3 | - | 16 | - | 2 | 8.33 |
| | | - | - | 22 | - | 3 | 12.48 |
| | | - | - | 20 | 4 | 2 | 8.33 |
| | | - | - | - | 44 | 1 | 4.16 |
| Range | | 0-8 | - | 6-22 | 0-44 | | |
| Mean | | 4.75 | - | 11.41 | 2.25 | | |
| 2 | 49 | 8 | - | 6 | - | 12 | 20.32 |
| | | 8 | - | 5 | 2 | 3 | 5.08 |
| | | 8 | - | 4 | 4 | 5 | 8.47 |
| | | 7 | - | 8 | - | 10 | 16.94 |
| | | 6 | - | 10 | - | 5 | 8.47 |
| | | 6 | - | 8 | 2 | 8 | 13.52 |
| | | 3 | - | 16 | - | 7 | 11.83 |
| | | - | - | 22 | - | 9 | 15.25 |
| Range | | 6-8 | - | 4-22 | 0 - 4 | | |
| Mean | | 5.57 | - | 10.01 | 0.71 | | |
| 3 | 46 | 8 | - | 6 | - | 15 | 32.65 |
| | | 8 | - | 4 | 4 | 3 | 6.53 |
| | | 6 | - | 9 | 2 | 3 | 6.53 |
| | | 6 | - | 10 | 2 | 4 | 8.68 |
| | | 5 | - | 12 | - | 6 | 13.04 |
| | | 4 | - | 14 | - | 6 | 13.04 |
| | | 3 | - | 16 | - | 4 | 8.68 |
| | | - | - | 22 | - | 5 | 10.85 |
| Range | | 0-8 | - | 4-22 | 0-4 | | |
| Mean | | 5.47 | - | 10.84 | 0.56 | | |

Table - 144

Chiasma frequency at metaphase - I in induced tetraploids of Alyosia scarabaeoides

| Genera- tion | No. of cells studied | VI | V | No. of Quadri- valents with 3xmata 4xmata | No. of tri- valents | No. of bivalents with 2xmata 1xma | No. of univa- lents | No. of total xmata per cell |
|---------------------------|----------------------------|----|---|--|------------------------|---|------------------------|-----------------------------------|
| C ₀ | 60 | 3 | 1 | 78 | 210 | 483 | 150 | 195 2220 37.0 |
| C ₁ Plant 1 | 24 | - | - | 12 | 102 | 204 | 70 | 54 922 38.41 |
| Plant 2 | 59 | - | - | 28 | 301 | 388 | 203 | 42 2267 38.42 |
| Plant 3 | 46 | - | - | 2 | 250 | 400 | 99 | 26 1905 41.41 |

Table - 145

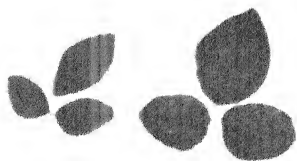
Chromosome distribution at anaphase - I in induced tetraploids of Atylosia scarabaeoides
(% in parentheses)

| Generation | No. of cells studied | Anaphase - I | | Sporad Stage No. of Tetrad nuclei. cells studied | pollen fertility % | fertile pollen size | |
|----------------|----------------------|------------------|----------------------|--|--------------------|---------------------|---------------------------|
| | | Equal separation | unequal distribution | | | Range (μ) | Mean (μ) |
| C ₀ | 45 | 44 (97.88) | - | 1 (2.22) | 88 (97.77) | 2 (2.22) | 72.11 33 - 48 36.00 |
| C ₁ | 43 | 40 (92.80) | - | 3 (6.97) | 92 (96.6) | 3 (3.15) | 89.2 36 - 45 37.8 |
| Plant No. 1 | | | | | | | 63 44 80 |
| Plant No. 2 | 61 | 59 (96.72) | 2 (3.27) | - | 93 (97.65) | 2 (2.15) | 91.5 36 - 45 37.5 |
| Plant No. 3 | 35 | 35 (100) | - | - | 82 (100) | - | 90.6 36 - 45 38.7 |

PLATE - 22 (Induced tetraploid of A. scarabaeoides)

- Fig. 1. Leaves of diploid and tetraploid (Left to Right)
- Fig. 2. Pods of diploid and tetraploid (Left to Right)
4 in each case.
- Fig. 3. Stomata of diploid (X 600)
- Fig. 4. Stomata of tetraploid (X 600)
- Fig. 5. 1 VI + 6 IV's + 7 II's at Metaphase-I (C_0)
(X 1500)
- Fig. 6. 10 IV's + 2 II's at Metaphase-I (C_0) (X 1500)
- Fig. 7. 22 II's at Metaphase-I (C_0) (X 1500)
- Fig. 8. 44 I's at Metaphase-I (C_0) (X 1500)
- Fig. 9. Pollen grains of diploid, (X 600) (C_0)
- Fig. 10. Pollen grains of tetraploid, (X 600) (C_0)
- Fig. 11. Micronuclei and hexad (C_0) (X 400)
- Fig. 12. 8 IV's + 5 II's + 2 I's at Metaphase-I (C_1)
(X 1500)
- Fig. 13. 44 somatic chromosomes at Metaphase-I (C_1)
(X 1500)

PLATE - 22

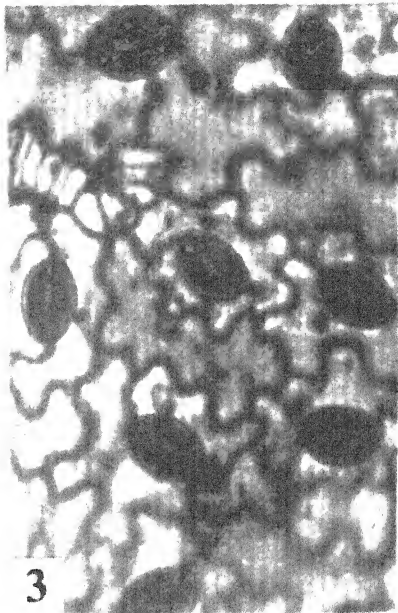


1

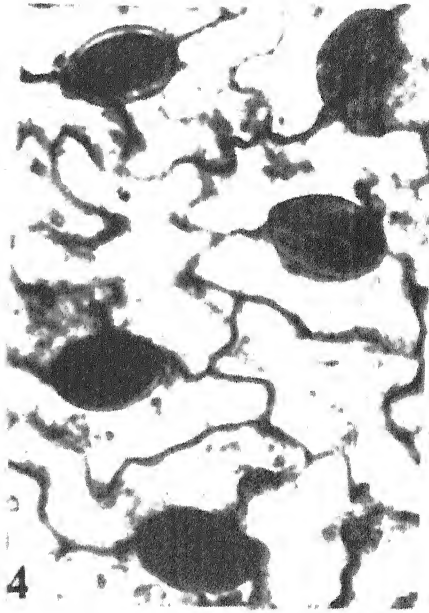
2



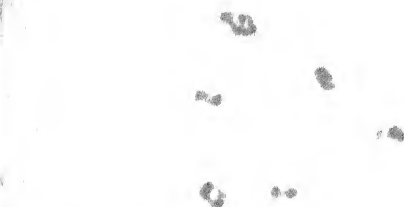
2



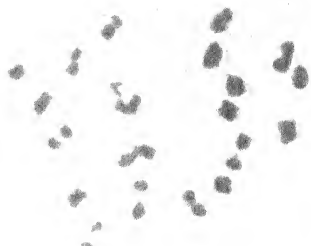
3



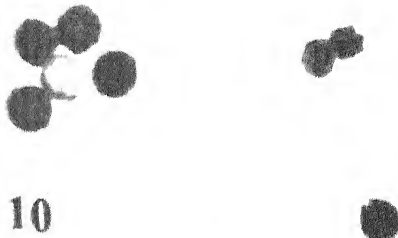
4



5



7



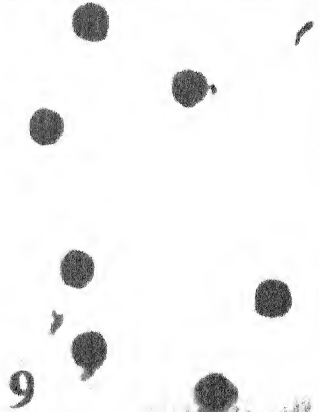
10



6



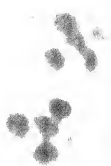
8



9



12



11



13

percentage of cells (32.65) were noticed with chromosomal association of 8 IVs + 6 IIs in 32.65 per cent cells. Bivalents and univalents ranged from 4 to 22 and 0-4 with 10.84 and 0.56 per cell respectively. Maximum number of 22 bivalents, and 4 univalents were observed in 10.85 and 6.53 per cell respectively (Table-143). Chiasma frequency at metaphase-I was 41.41 per cell (Table-144). At anaphase-I equal separation of chromosomes to the poles was noticed in all the cells studied (Table-145). At the sporad stage, regular tetrad formation was observed in all the cells studied. Pollen fertility percentage was 90.6 and fertile pollen size ranged from 36 to 45 μ with 38.7 μ mean diameter.

Observations on the effects of colchicine on *Caianus caian*.

a) Seed germination:

The effects of colchicine on seed germination at different concentrations and durations of treatments are as follows:

All the seeds germinated after the treatments with 0.05% colchicine for 4, 6 and 8 hours (Table-146). When the treatment prolonged to 24 hours, out of the total seeds, only 30.0% seeds got germinated. Application of 0.1% colchicine for 4, 6 and 8 hours resulted in germination of all the seeds through prolonged treatment for 24 hours exhibited only 20.0% seed germination. Treatments of 0.2% colchicine solution for 2 and 4 hours had no effect on seed germination, while in the increased duration of treatments (6 and 8 hours) germination percentage was recorded to be 90.0 and 50.0 respectively.

b) Plant survival:

The effects of colchicine on plant survival was studied in the experiments on seed and seedling treatments.

The seedlings could not emerge from the colchicine treated seeds in all the treatments, hence no plant could be obtained. Seedlings when immersed in 0.05% aqueous colchicine solution for the period of 4, 6 and 8 hours percentage survival of seedlings were 80.0, 40.0 and 20.0 respectively. Seedlings immersed in 0.1% colchicine solution for periods of 4, 6 and 8 hours showed 10.0, 6.66 and 3.33 per cent survival respectively (Table-146). The highest concentration of colchicine (0.2%) when used for 2 hours, 50.0% plant survival was observed, whereas, the other treatments for 4, 6 and 8 hours proved to be toxic.

Apical buds of seedlings treated with colchicine showed differential survival of seedlings at different concentrations and durations. After the treatments with 0.05% colchicine 8 hours a day for one, two and three days, 80.0%, 60.0 and 48.0 per cent seedlings survived respectively. When 0.1% colchicine solution applied for 8 hours a day for one, two and three days, percentage seedling survival were 53.3, 13.3 and 9.99 respectively. The highest concentration (0.2%) of colchicine solution used for 8 hours a day resulted in 2.66% seedling survival. When the same concentration used for 8 hours a day for two and three days, no seedling could survive thereafter.

c) Production of polyploid:

Chromosome doubling was successfully induced when the apical buds were treated through the absorbent

cotton plug soaked in 0.2% aqueous colchicine solution for 8 hours a day for one day.

Studies on induced tetraploid of *Cajanus cajan*.

a) Morphology:

Comparative morphological characters of diploid and induced tetraploid of *Cajanus cajan* are summarised in Table-147. Their details are as follows:

1) Seedling, branches and stem height:

After treatment of the apical buds of the seedlings, the first pair of leaves gradually become darker green in colour and thicker than the untreated ones. The induced tetraploid of *Cajanus cajan* in C_0 generation, showed less number of primary and secondary branches in comparison to the diploid. The number of primary and secondary branches in tetraploid plants were 2 and 3, while diploid showed average 6 primary and 14 secondary branches. The C_0 plant of *Cajanus cajan* showed reduced plant height (56.0 cm) as compared to diploid (118 cm).

In the C_1 generation, first pair of simple leaves was darker green in colour and thicker than the diploid. The number of primary and secondary branches were 4 and 6 respectively and the stem height was 115 cm.

2) Days to flowering and maturity:

Delayed flowering and maturity were observed in tetraploid in contrast to diploids.

After, sowing, the C_0 plant took 115 days for bud initiation and 138 days for 50% flowering. Whereas the

diploid plant took on an average, 92 and 105 days for bud initiation and 50% flowering. Average number of days taken by buds for full development into flowers in diploid and C_0 plant were 12 and 16 respectively and the duration between pod initiation to pod maturity were 40 and 30 days in tetraploid and diploid respectively. Days to 50% pod maturity were recorded to be 211 and 175 in C_0 and diploid respectively.

In C_1 plant, days from sowing to bud initiation and days from sowing to 50% flowering were 105 and 131 respectively, and from bud to full development into flower 14, for pod initiation to maturity 38. Days to 50% pod maturity was observed to be 207 in this plant.

3) Leaf:

The leaves of C_0 plant were thicker and darker green in colour in contrast to the diploid. Marked increase in length and breadth of leaves (Plate-23; Fig. 1) in C_0 plant was noticed. The average central leaf let length and breadth were 5.3 cm and 2.0 cm as against 4.0 cm length and 1.5 cm breadth in diploid. Similarly the length of petiole was 2.3 cm in tetraploid and 2.1 cm in diploid. The surface of leaves of diploid as well as tetraploid was non-hairy.

In C_1 plant, average length and breadth of Central leaf lets were 5.5 cm and 2.2 cm respectively and the average petiolar length was 2.6 cm. The leaves of C_1 plant were also darker green in colour and thicker as compared to diploid. The leaf surface was non-hairy in this plant.

4) Flower:

The C_0 plant produced larger flowers as compared to diploids. The size of the standard petal of C_0 plant was

2.88 cm² as against 2.10 cm² of diploid. On an average, the length of style was found to be 1.8 cm in tetraploid and 1.5 cm in diploid.

In C₁ plant, the size of the standard petal was 3.06 cm² and 1.8 cm stylon length.

5. Pods:

The induced tetraploid of Caianus caian (C₀) showed 4.0% pod setting as against 30.0% in diploid plants. In C₁ plant pod setting was increased (4.0%) as compared to C₀ plant.

In tetraploid plant (C₀) reduced pod size was recorded in comparison to diploid (2.88 cm² in tetraploid (C₀) and 3.76 cm² in diploid). Number of chambers per pod was 2.0 in tetraploid and 3.1 in diploid and the number of seeds per pod was 1.0 and 2.3 in induced tetraploid and diploid respectively. Pods of diploids as well as tetraploid were non-hairy.

In C₁ plant average pod size was 3.12 cm² and pod thickness was 0.78 cm. All the pods of C₁ plant were non hairy. In this plant average number of chambers per pod and number of seeds per pod were 2.4 and 1.1 respectively.

6) Seeds:

Ovule fertility percentage was 87.0, 25.0 and 50.0 in diploid, C₀ and C₁ plants respectively. The seeds of C₀ plant were bold in comparison to the diploid plant. Average seed thickness in C₀ was 0.50 cm while it was 0.41 cm in the diploid. In C₁ generation, average seed thickness was observed to be 0.52 cm.

7. Stomata:

An increase in the size of stomata of tetraploid plant over the diploid was noticed (Plate-23; Fig. 2,3). The average length and breadth of stomata in C_0 plant was 21 μ and 18 μ respectively as against 15 μ length and 12 μ breadth in diploid. The tetraploid plant exhibited reduction in number of stomata per unit area (4.5) as compared to diploid (6.0).

In C_1 plant, the reduction in number of stomata per unit area was observed with mean value of 5.0 stomata per unit area. The average stomatal length and breadth were 16.8 μ and 17.5 μ respectively.

b) Cytology (C_0).

Mitosis:

Mitotic studies in root tip cells of colchicine treated seeds of Cajanus cajan have shown different ploidy levels as 4n and 8n (Table-148), at different concentrations of 0.025% colchicine solution used for 6 hours brought about only condensation of chromosomes. While at 0.05% concentration and 6 hours duration, 28.5% cells showed tetraploidy and 9.5% cells octoploidy. The remaining 60.4% cells were diploid. In the treatment with 0.1% for 6 hours duration, 2n, 4n and 8n ploidy levels were observed in 5.5, 28.5 and 9.5 per cent cells respectively. When 0.2% colchicine solution applied for two hours, 22, 44 and 88 chromosomes were observed in 7.50, 11.1 and 81.4% cells respectively. At 0.2% concentration and 4 hours duration, increase in cells with 4n and 8n chromosome numbers were observed in 20.0 and 72.0 per cent cells respectively. When 0.2% colchicine solution was used for 6 hours and 8 hours

durations, gradual increase in cells with $4n$ and $8n$ chromosomes were recorded (Table-148). When 0.2% colchicine used for 8 hours for 44 and 88 chromosomes were observed in 20.0% and 80.0% cells respectively.

Meiosis: (C_0 plant).

Meiotic study in C_0 plant revealed various chromosomal associations as pentavalent, quadrivalent (Fig. 4,5), trivalents and bivalent at metaphase-I (Table-149). Pentavalents ranged from 0-1 with 0.04 per cell and presence of one pentavalent was observed in 4.34% cells. At metaphase-I, quadrivalents and trivalents ranged from 2-8 and 0-3 with 4.60 and 0.21 per cell respectively. Maximum number of 8 quadrivalents and 3 trivalents were observed in 34.72% and 4.34% cells respectively. Bivalents and univalents ranged from 6-17 and 0-3 with 11.78 and 0.43 per cell respectively (Table-149). Chiasma frequency at metaphase-I was 40.45 per cell (Table-150). At anaphase-I, laggards and unequal distribution of chromosomes (Plate-23; Fig. 6) were observed in 3.84 and 1.92 per cent cells respectively. However in the remaining 94.08% cells normal separation of chromosomes to the poles was observed (Table-151). At spored stage, regular tetrad formation was observed in 89.41% cells except in 4.70% cells wherein micronuclei were formed.

Pollen fertility was 82.7% and fertile pollen size (Plate-23; Figs. 8,9) ranged from 39-48 μ with 44.7 μ mean diameter in induced tetraploid of C. caian while in diploids it ranged from 36-45 μ .

Cytology: (C_1).

a) Mitosis:

Fourty four somatic chromosomes were counted in

the root tip cells at metaphase (Plate-23; Fig. 12).

b) Meiosis:

In this plant, meiotic study revealed formation of quadrivalents, trivalent, bivalents and univalents at metaphase-I (Table-149). The quadrivalents ranged from 0-8 with an average 3.32 per cell. Maximum number of 8 quadrivalents were observed in 11-76% cells. Bivalents and univalents, at metaphase-I, ranged from 6-22 and 0-4 with 16.97 and 0.64 per cell respectively. Trivalents ranged from 0-1 with 0.05 per cell and presence of one trivalent was observed in 5.88% cells. Chiasma frequency at metaphase-I was 41.32 per cell (Table-150). At anaphase-I, laggards were observed in 7.14% cells and in remaining 92.85% cells equal separation of chromosomes to the poles was observed.

At sporad stage, micronuclei formation was noticed in 6.31% cells and in 93.45% cells regular tetrad formation was observed.

Pollen fertility was 86.3 and fertile pollen size ranged from 39 to 46 μ with 44.4 μ mean diameter.

Table - 146

(SWT coll.)

Effects of calchicine on seed germination and plant survival in *Cajanus cajan* (SWT coll.)

No. of seeds treated in each case were - 10; figures in parentheses are %.

| Seed treatment | | Seedling treatment (immersion) | | Seedling treatment (col. through absorbent) | | Cotton plug method | | Tetra- | |
|---------------------------|-------------------------|--------------------------------|---------------------------|---|-------------------------------------|---------------------------|----------------------------------|-------------------------------------|-------------------|
| Concen- tration (%) | Dura- tion (hrs.) | Seeds germi- nated | Concen- tration (%) | Dura- tion (hrs.) | No. of seed- lings treated | Concen- tration (%) | Dura- tion (hrs./ days) | No. of seed- lings treated | Survived ploid |
| 0.05 | 4 | 10 (100) | 0.05 | 4 | 20 | 0.05 | A ₁ | 25 | 20 (80.0) |
| 0.05 | 6 | 10 (100) | 0.05 | 6 | 20 | 0.05 | A ₂ | 25 | 15 (60.0) |
| 0.05 | 8 | 10 (100) | 0.05 | 8 | 20 | 0.05 | A ₃ | 25 | 12 (48.0) |
| 0.05 | 24 | 3 (30.0) | 0.1 | 4 | 30 | 0.1 | A ₁ | 30 | 16 (53.3) |
| 0.1 | 4 | 10 (100) | 0.1 | 6 | 30 | 0.1 | A ₂ | 30 | 4 (13.3) |
| 0.1 | 6 | 10 (100) | 0.1 | 8 | 30 | 0.1 | A ₃ | 30 | 3 (9.99) |
| 0.1 | 24 | 2 (20.0) | 0.2 | 2 | 20 | 0.2 | A ₁ | 75 | 2 (2.66) |
| 0.2 | 2 | 10 (100) | 0.2 | 4 | 20 | 0.2 | A ₂ | 50 | 0 |
| 0.2 | 4 | 10 (100) | 0.2 | 6 | 20 | 0.2 | A ₃ | 35 | 0 |
| 0.2 | 6 | 5 (50.0) | 0.2 | 8 | 20 | 0.2 | | | |
| 0.2 | 8 | 1 (10.0) | 0.2 | 8 | 20 | 0.2 | | | |

A₁ = 8 hours - One day; A₂ = 8 hours 2 days; A₃ = 8 hours - 3 days.

Table - 147

Comparative morphological observations in diploid and induced tetraploid of Cajanus cajan (SNT coll.)

| Characters | <u>C. cajan</u> 2x | <u>C. cajan</u> 4x (C ₀) | <u>C. cajan</u> 4x (C ₁) |
|---|------------------------|---|---|
| No. of primary branches | 6 | 2 | 4 |
| No. of secondary branches | 14 | 3 | 6 |
| Central leaflet: surface (L x B) cm | Non-hairy 4.0 x 1.5 | Non-hairy 5.3 x 2.0 | Non-hairy 5.5 x 2.2 |
| length of petiole (cm.) | 2.1 | 2.5 | 2.6 |
| Height of plant (cm.) | 118 | 56.0 | 115 |
| Days from sowing to bud initiation | 92 | 115 | 105 |
| Days from sowing to flowering | 105 | 138 | 131 |
| Days between bud to flowers | 12 | 16 | 14 |
| Days between pod initiation to maturity | 36 | 40 | 38 |
| Size of the standard petal (L x B) cm. | 1.5 x 1.4 | 1.8 x 1.6 | 1.8 x 1.7 |
| Length of style (cm.) | 1.5 | 1.8 | 1.8 |
| Pod (L x B) cm. | 4.7 x 0.8 | 3.6 x 0.8 | 3.9 x 0.8 |
| Thickness of pod (cm.) | 0.700 | 0.50 | 0.78 |
| Hairs on mature pod | Absent | Absent | Absent |
| No. of chambers per pod | 3.1 | 2.0 | 2.4 |
| No. of seeds per pod | 2.3 | 1.0 | 1.1 |
| Thickness of seed (cm.) | 0.41 | 0.50 | 0.52 |
| Days to maturity | 175 | 211 | 207 |
| pod set (%) | 30.0 | 4.0 | 9.0 |
| Ovule fertility (%) | 87.0 | 25.0 | 50.0 |
| Stomata: frequency (L x B) μ | 6.0 15 x 12 | 4.50 21 x 18 | 5.0 16.8 x 17.5 |

Table - 148

Effects of Colchicine on somatic chromosomes of Calanus calan (SNT Coll.)
(% in parentheses)

| Concen- tration % | Concen- tration (hours) | No. of cells studied | PLOIDY LEVEL AT META PHASE | | | |
|-------------------------|-------------------------------|----------------------------|----------------------------|--------------|--------------|-----|
| | | | 2n | 4n | 8n | 16n |
| 0.025 | 6 | 50 | 50 (100) | - | - | - |
| 0.05 | 6 | 51 | 31 (60.4) | 15 (28.5) | 5 (9.5) | - |
| 0.1 | 6 | 90 | 5 (5.5) | 81 (89.1) | 4 (4.4) | - |
| 0.2 | 2 | 27 | 22 (81.4) | 3 (11.1) | 2 (7.5) | - |
| " | 4 | 25 | 2 (8.0) | 5 (20.0) | 18 (72.0) | - |
| " | 6 | 30 | - | 16 (19.8) | 24 (79.2) | - |
| " | 8 | 25 | - | 5 (20.0) | 20 (80.0) | - |

Table - 149

Chromosome associations at Metaphase - I in induced tetraploids of Cajanus cajan (SNT Coll.)

| Gene- ration | No. of cells studied | Chromosomal associations at M-I | | | | | Frequency | Per cent |
|-----------------|----------------------------|------------------------------------|------|------|-------|------|-----------|-------------|
| | | V | IV | III | II | I | | |
| C ₀ | 46 | - | 8 | - | 6 | - | 16 | 34.72 |
| | | - | 5 | - | 12 | - | 12 | 26.04 |
| | | - | 6 | 1 | 11 | 3 | 4 | 8.69 |
| | | 1 | 4 | 3 | 7 | - | 2 | 4.34 |
| | | - | 3 | - | 16 | - | 8 | 17.39 |
| | | - | 2 | - | 17 | 2 | 4 | 8.69 |
| Range | | 0-1 | 2-8 | 0-3 | 6-17 | 0-3 | | |
| Mean | | 0.04 | 4.60 | 0.21 | 11.78 | 0.43 | | |
| C ₁ | 34 | - | 8 | - | 6 | - | 4 | 11.76 |
| | | - | 5 | - | 12 | - | 6 | 17.64 |
| | | - | 4 | 1 | 11 | 3 | 2 | 5.88 |
| | | - | 4 | - | 14 | - | 5 | 14.70 |
| | | - | 3 | - | 16 | - | 5 | 14.70 |
| | | - | 2 | - | 18 | - | 4 | 11.76 |
| | | - | 2 | - | 16 | 4 | 3 | 8.82 |
| | | - | 2 | - | 17 | 2 | 2 | 5.88 |
| | | - | - | - | 22 | - | 3 | 8.82 |
| Range | | - | 0-8 | 0-1 | 6-22 | 0-4 | | |
| Mean | | - | 3.32 | 0.05 | 16.99 | 0.64 | | |

Chiasma frequency in induced tetraploids of *Cajanus cajan* at Metaphase - I.

| Genera- tion | No. of cells studied | No. of penta- valents | No. of quadrivalents with 3xmata | No. of triva- lents | No. of bivalents with 2xmata | No. of univa- lents | Total chiasmata xmata per cell |
|-----------------|----------------------------|-----------------------------|---|---------------------------|------------------------------------|---------------------------|-----------------------------------|
| C ₀ | 46 | 2 | 10 | 10 | 457 | 85 | 20 1861 40.45 |
| C ₁ | 34 | - | 9 | 2 | 380 | 198 | 22 1805 41.32 |

Table - 151

Chromosome distribution at Anaphase - I in induced tetraploids of *Cajanus cajan*.

| Genera- tion | No. of cells studied | Equal distri- bution | Unequal distri- bution | Laggards | No. of cells studied | Sporad Stage | | Pollen ferti- lity % | Pentile pollen size | |
|-----------------|----------------------------|----------------------------|------------------------------|-------------|----------------------------|---------------|-------------------|-------------------------------|------------------------|-------------|
| | | | | | | Tetrad | Micro- nuclei. | | Range (n) | Mean (n) |
| C ₀ | 52 | 49 (94.08) | 1 (1.92) | 2 (3.84) | 80 | 76 (89.41) | 4 (4.70) | 82.7 | 39-49 | 44.7 |
| C ₁ | 28 | 26 (92.85) | - | 2 (7.14) | 95 | 89 (93.45) | 6 (6.31) | 86.3 | 39-45 | 44.4 |

(figures in parentheses are per cent)

PLATE - 23 (Induced tetraploid of G. cajan)

Fig. 1. Leaves of diploid and tetraploid (Left to Right)

Fig. 2. Stomata of diploid (C_0) (X 600)

Fig. 3. Stomata of tetraploid (C_0) (X 600)

Fig. 4. 3 IV's + 16 II's at Metaphase-I (C_0) (X 1500)

Fig. 5. 5 IV's + 12 II's at Metaphase-I (C_0) (X 1500)

Fig. 6. Laggaras and unequal distribution at Anaphase-I (18 = 2-24) (X 1500)

Fig. 7. Hexad with normal tetrads (C_0) (X 600)

Fig. 8. Pollen grains of diploid (C_0) (X 600)

Fig. 9. Pollen grains of tetraploid (C_0) (X 600)

Fig. 10. 4 IV's + 1 III + 11 II's + 3 I's at diakinesis (C_1) (X 1500)

Fig. 11. 2 IV's + 17 II's + 2 I's at diakinesis (C_1) (X 1500)

Fig. 12. 44 somatic chromosomes at Metaphase-I (C_1) (X 1500)

PLATE — 23

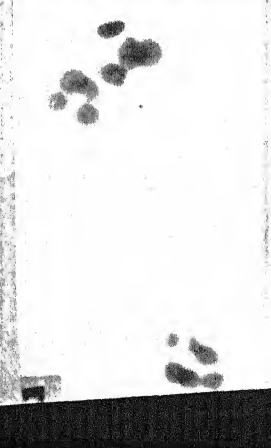
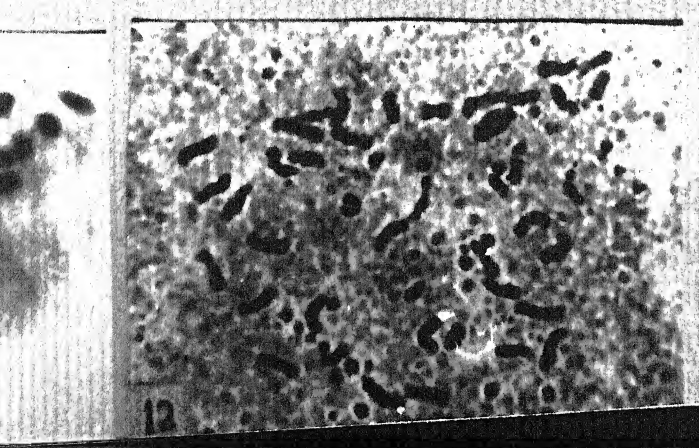
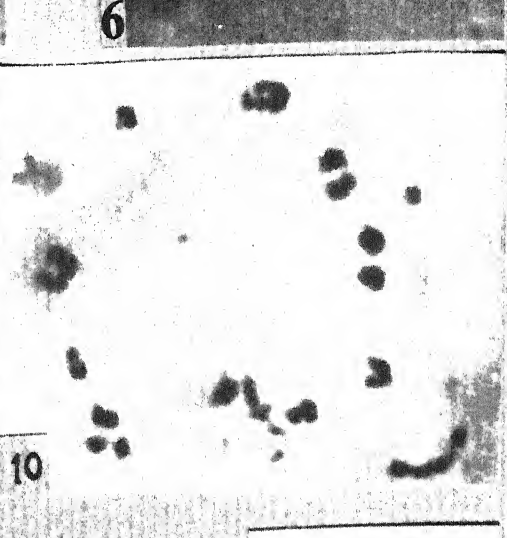
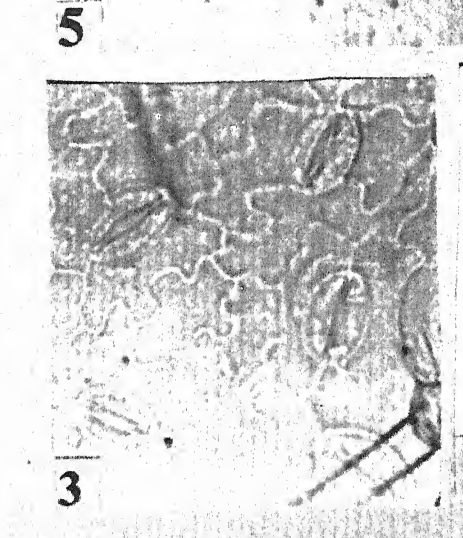
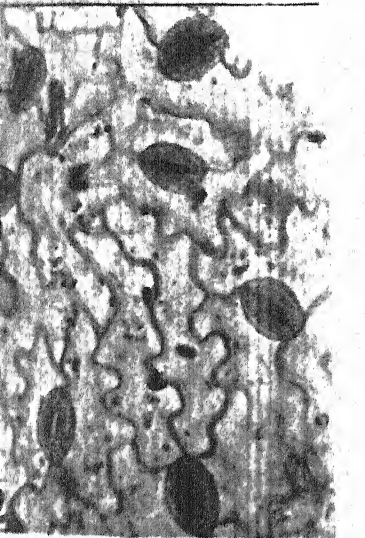
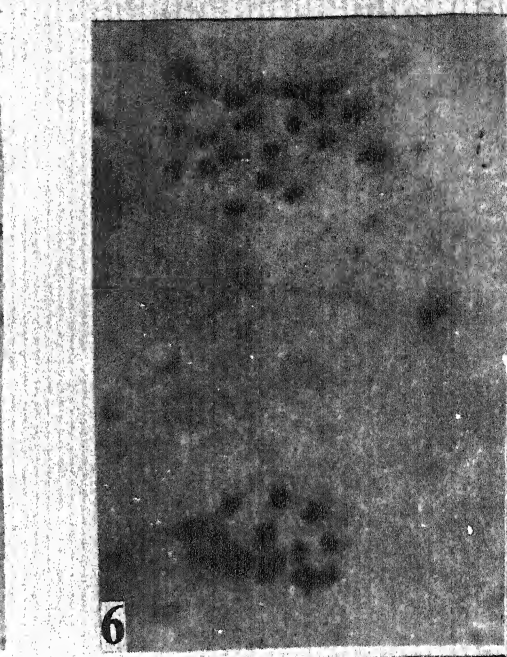
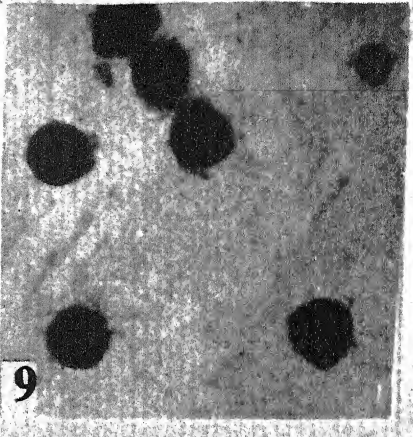
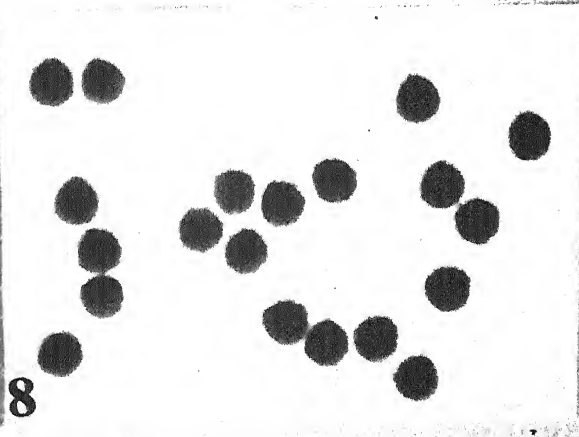
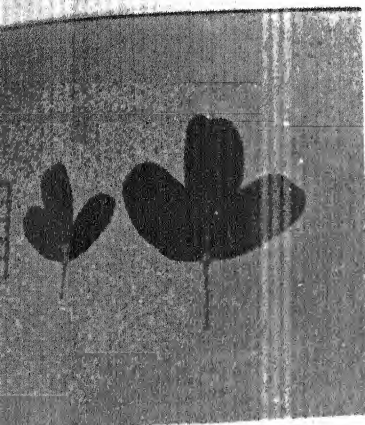


PLATE - 24 (Colchicine treated somatic chromosomes)

- Fig. 1. 22 chromosomes at (A. albicans) (X 1500)
- Fig. 2. 44 chromosomes at (A. albicans) (X 1500)
- Fig. 3. 88 chromosomes (A. albicans) (X 1500)
- Fig. 4. 44 chromosomes of A. scarab. at Metaphase (X 1500)
- Fig. 5. 44 Chromosome of A. cajanifolia at Metaphase (X 1500)
- Fig. 6. 88 Chromosomes of A. scarab. at Metaphase (X 1500)
- Fig. 7. 88 Chromosomes of A. cajanifolia at Metaphase (X 1500)
- Fig. 8. 44 Chromosomes of A. volubilis at Metaphase (X 1500)
- Fig. 9. 88 Chromatids of A. volubilis at late Metaphase (X 1500)
- Fig. 10. 88 Chromosomes of A. volubilis at early metaphase (X 1500)

10u

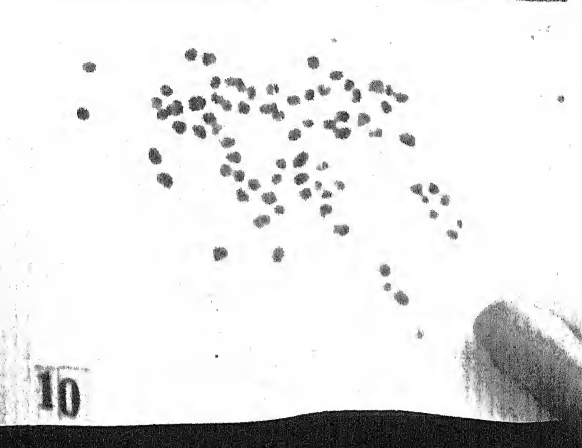
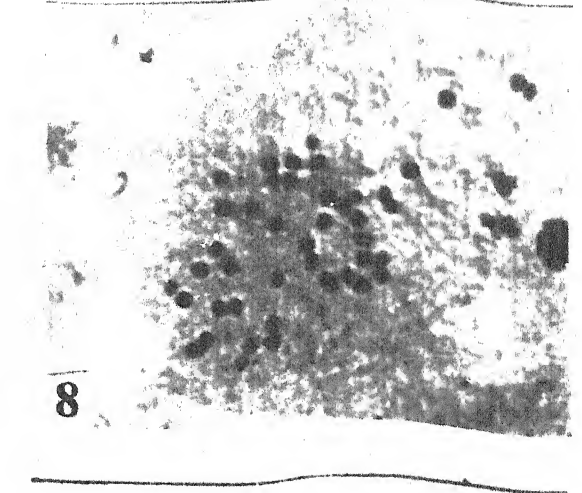
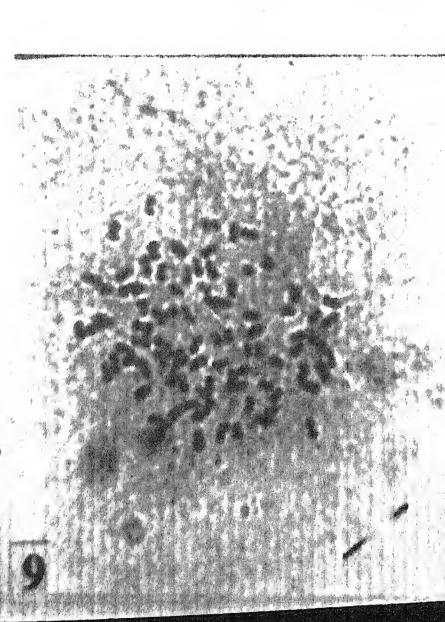
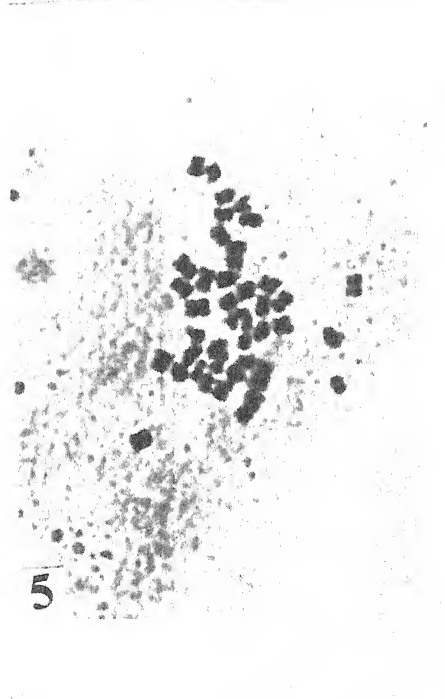
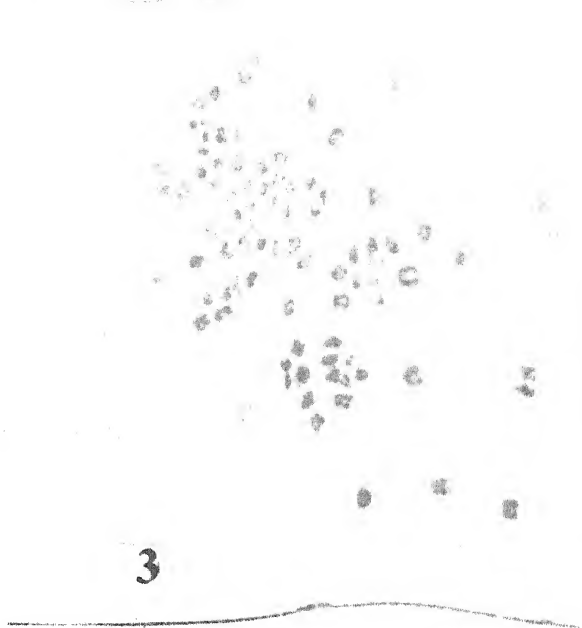


PLATE - 25 (colchicine treated somatic chromosomes)

- Fig. 11. 44 Chromosomes of A. platycarpa (X 1500)
- Fig. 12. 88 chromosomes of A. platycarpa (X 1500)
- Fig. 13. 16x chromosomes of A. platycarpa (X 1500)
- Fig. 14. 44 Chromosomes of A. lineata (X 1500)
- Fig. 15. 88 \times chromosomes of A. lineata (X 1500)
- Fig. 16. 22 Chromosomes of C. cajan (SNT Coll)
- Fig. 17. 44 Chromosomes of C. cajan (SNT Coll.)
- Fig. 18. 44 Chromosomes of C. cajan (ICP 8647) (X 1500)
- Fig. 19. 44 chromosomes of C. cajan (ICP 8647) (X 1500)

PLATE - 25

1.4

11

12

13

15

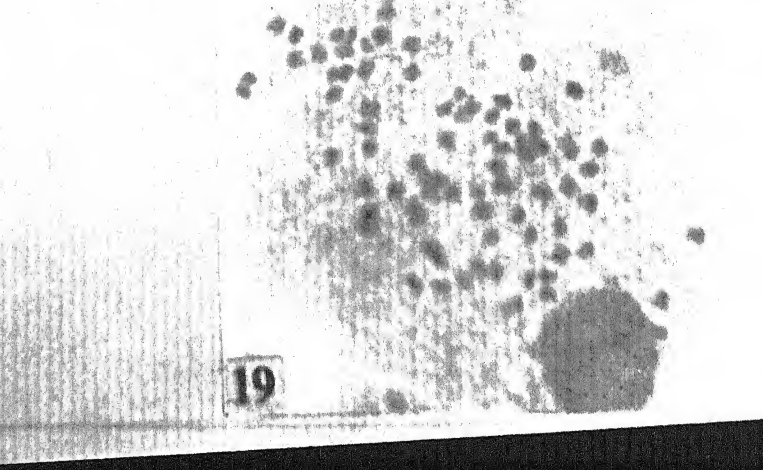
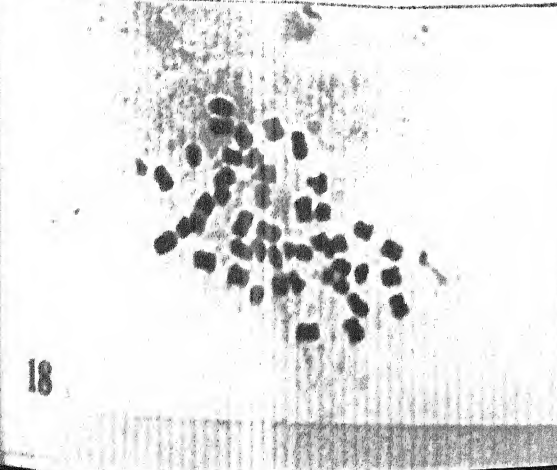
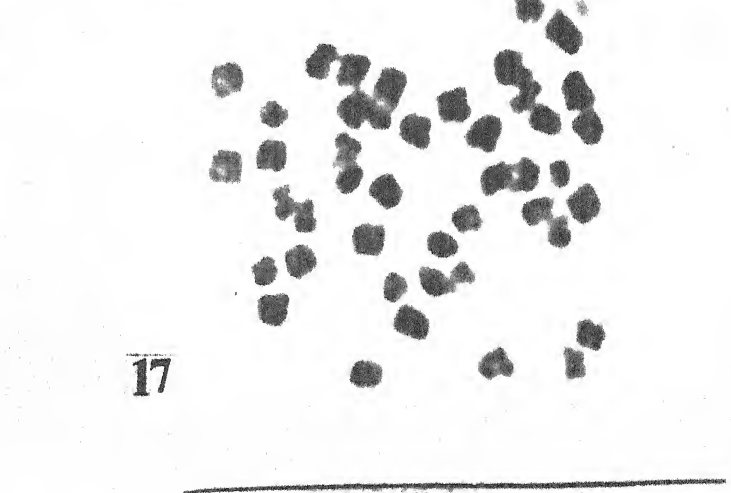
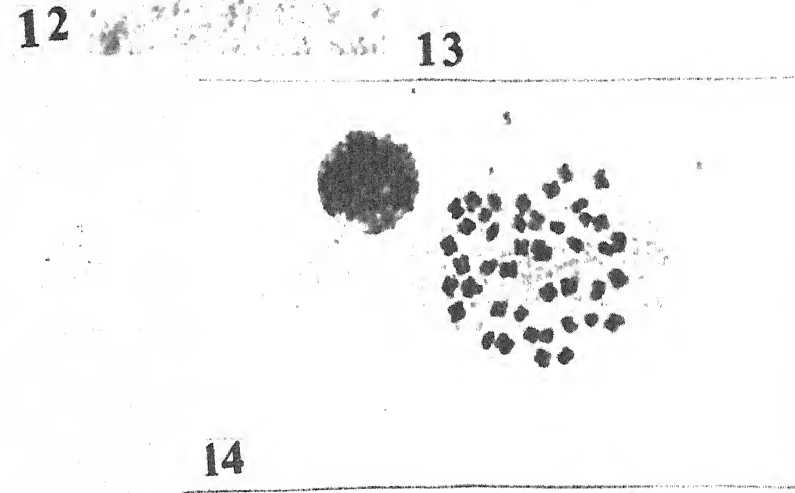
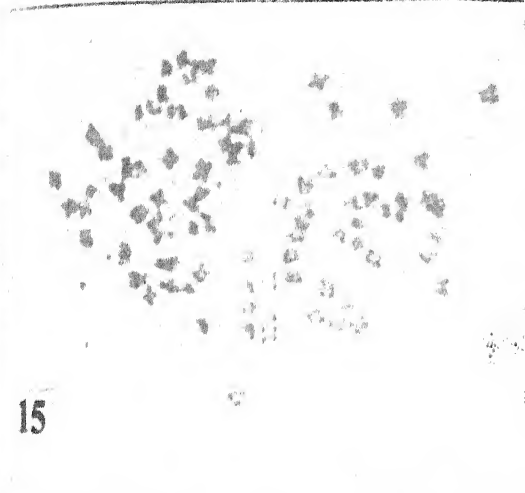
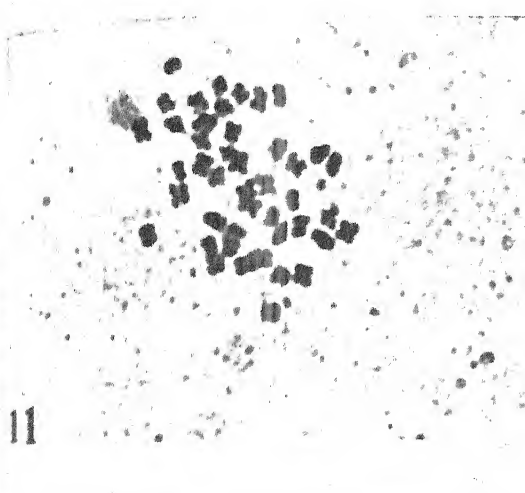
14

16

17

18

19



Effects of EMS on seed germination and plant survival in
Atylosia platycarpa.

Gradual reduction in the percentage of seed germination and plant survival was noticed with increase in concentration and duration of treatments. Observations on seed germination in petridishes, emergence of plumule in field and survival to maturity in 4 and 8 hours treatments, at different concentration are as follows:

4 hours treatment:

After the treatment with the lowest concentration of 0.2% EMS solution for 4 hours, 95.0 per cent seed germination, 90.0 per cent plumule emergence in field and 85.0 per cent plant survival to maturity was recorded (Table-152). At 0.4% concentration, seed germination, plumule emergence in the field and survival to maturity were 85.0, 80.0 and 75.0 per cent respectively. In the treatment with 0.6% and 0.8% EMS solution reduction in the percentage of seed germination was plumule emergence and survival to maturity was noticed (Table-152). When the highest concentration of 1.0% solution was used only 30.0 per cent seeds could germinate and perhaps plumules could not emerge due to toxic effects of the chemical. However no seedling was raised.

8 hours treatment:

At 0.2% concentration, 90.0 per cent seeds germinated, 85.0 per cent plumule emerged and 80.0 per cent plants survived. 0.4% EMS solution when used for the periods of 8 hours, percentage seed germination reduced to 80.0, plumule emergence 70.0 and plant survival to 60.0 respectively (Table-152). In the treatment with 0.6 and 0.8 per cent EMS, seed germination percentage was recorded as 75.0 and 65.0 respectively. A subsequent

reduction in plumule emergence in the field and plant survival to maturity was noticed (Table-152). At the highest concentration of EMS solution (1.0%) only 20.0 per cent seed germination was recorded. Plumules could not emerge after such a treatment.

Morphological observations in EMS treated plants of *Atylosia platycarpa*.

Studies on different morphological characters were recorded in EMS treated plants and compared with those of control (Table-153). Morphological observations at various concentrations and durations are as follows.

a) 4 hours treatment:

(i) 0.2%:

M_1 plants showed 35.5 cm average plant spread. On an average, the number of primary and secondary branches were 4.2 and 6.1 respectively. Days to 50 per cent flowering and maturity resembled control plant. Number of pods per plant and seeds per pod was 28.1 and 2.3 respectively.

In M_2 plants, 38.4 on average plant spread was recorded. Number of primary and secondary branches were 6.3 and 8.2 respectively. Days to 50% flowering and maturity were nearer to those of control plants (Table-153).

(ii) 0.4%:

M_1 plants showed on an average 34.2 cm plant spread and 5.2 and 7.5 primary and secondary branches. Length and breadth of central leaflet was 4.1 cm and 4.2 cm respectively. Days to 50% flowering and maturity were nearer to control plants. An average number of pods per plant and seeds per pod was 22.3 and 1.7 respectively.

In M_2 plants, average plant spread was 37.1 cm. Number of primary and secondary branches were 5.6 and 8.0 respectively. The number of pods per plant and seeds per pod, on an average were 32.6 and 22.2 respectively.

0.6%:

In this treatment, average plant spread was 36.4 cm. The number of primary and secondary branches were 7.5 and 7.6 respectively. Average length and breadth of central leaf let was 5.0 cm and 4.6 cm respectively. Number of pods per plant and seeds per pod was 22.5 and 2.5 respectively. In M_2 plants increase in pods per plant and seeds per pod over M_1 plants was observed (Table-153).

0.8%:

Average plant spread was 25.2 cm. The number of primary and secondary branches were 4.1 and 5.1 respectively. Average leaf length and breadth was 4.9 cm and 4.0 cm respectively. Days to 50% flowering and maturity in M_1 plants were nearer to control. Number of pods per plant and seeds per pod were 7.5 and 1.1 respectively.

In M_2 plants, 35.1 cm average plant spread was noticed. The number of primary and secondary branches were 5.0 and 5.3 respectively. Number of pods per plant and seeds per pod were 27.5 and 2.5 respectively.

8 hours treatment:

0.2%:

In M_1 plants, 37.1 cm average plant spread was recorded (Table-153). The number of primary and secondary branches were 6.1 and 7.1 respectively. Days to 50% flowering, and maturity were nearer to those of control

plants. Average length and breadth of central leaflet was 4.2 cm and 3.9 cm respectively. Number of pods per plant and seeds per pod on an average were 30.1 and 1.3 respectively.

In M_2 plants, average plant spread was 32.3 cm and number of primary and secondary branches were 6.1 and 7.1 respectively. Average leaf length and breadth was 4.5 cm and 4.1 cm respectively. Days to 50% flowering, and maturity were nearer to those of control (Table-153). Number of pods per plant and seeds per pod were 34.1 and 1.9 respectively.

0.4%:

M_1 plants showed average 30.4 cm, plant spread. The average number of primary and secondary branches were 5.7 and 8.2 respectively. Leaf length and breadth was 4.0 and 4.0 cm. Delayed in 50% flowering and maturity was recorded (Table-153). On an average, pods per plant and seeds per pod were 30.2 and 1.7 respectively.

In M_2 plants, 33.3 cm average plant spread was noticed. On an average number of primary and secondary branches were 6.0 and 8.3 respectively. Days to 50% flowering, and maturity were nearer to those of control plants. Average number of pods per plant and seeds per pod were 38.3 and 2.2 respectively.

0.6%

In M_1 generation on an average, plant spread was 32.3 cm and number of primary and secondary branches were 5.4 and 7.5 respectively. M_1 plants showed 4.2 cm average length and 4.1 cm average breadth of central leaf let

respectively. Days to 50% flowering and maturity ^{were} 63 and 120 as against 58 and 126 in control plants. The average number of pods per plant and seeds per pod were 14.4 and 1.5 respectively.

In M_2 plants average plant spread of 38.9 cm was recorded and the number of primary and secondary branches were 5.7 and 7.9 respectively. Days to 50% flowering and maturity were nearer to those of control plants. Average leaf length and breadth were observed to be 4.5 cm to 4.3 cm respectively. Number of pods per plant and seeds per pod were 26.6 and 2.5 respectively.

0.8%:

The M_1 plants exhibited 31.4 cm average plant spread. The number of primary and secondary branches were 5.3 and 6.5 respectively. On an average, leaf length and breadth was 4.1 and 4.0 cm respectively. Days to 50% flowering and maturity were 65 and 132 as against 58 and 126 in control plants. The average number of pods per plant and seeds per pod were 9.5 and 1.0 respectively.

In M_2 plants, average plant spread was 34.5 and number of primary and secondary branches were 5.8 and 7.1 respectively. Days to 50% flowering and maturity were nearer to those of control plants. An increase in pods per plant and seeds per pod over M_1 plants was recorded (Table-153).

Cytology (M_1).

Mitosis:

Observations made in the root tip cells of M_1 seeds revealed stickiness, clumping and chromosome breakage

(Plate-26). Chromosomal abnormalities as observed during mitotic division are summarised in Table-154. Details are as follows:

4 hours treatment:

Treatment with 0.2% EMS Solution used for 4 hours showed no cytological effects. When 0.4% EMS solution was used for 4 hours chromosome stickiness and clumping was observed in 2.0% and 4.0% cells respectively. At both the above concentration equal anaphasic separation of chromosomes was recorded. At 0.6% concentration increase in the percentage of cells showing sticky chromosomes were recorded (Table-154). When 0.8% EMS solution was used for 4 hours chromosome break age was noticed in 2.0 per cent cells. The highest concentration of EMS (1.0%) showed chromosome breakage (Plate-26; Fig. 1) in 6.0 per cent cells. At anaphase-I, bridge (Plate-26; Fig. 3) with fragment and without fragment was noticed in 2.0 and 4.0 per cent cells respectively (Table-154).

8 hours treatment:

Treatment with 0.2% EMS solution showed no cytological effects. When 0.4% solution used for 8 hours chromosome stickiness and clumping was observed in 4.0 and 6.0 per cent cells respectively. At anaphase no abnormality was recorded. Treatment with 0.6% EMS solution increased in stickiness and clumping of chromosomes (Table-154). When 0.8% EMS solution was used, chromosome break age was noticed in 4.0 per cent cells and subsequent increase in stickiness and clumping of chromosomes was also recorded (Table-154). The highest concentration of EMS solution (1.0%) revealed chromosome breakage (Plate-26; Fig. 2) in 8.0 per cent cells.

Meiosis (M₁ plants):

Meiotic studies in M₁ plants revealed quadrivalents, trivalents, bivalents and univalents (Plate-26). Varying chromosomal configurations were noticed at different concentrations and durations of treatments (Table-155). The detail observations are as follows.

4 hours treatment:(i) 0.2%:

At metaphase-I, ring and rod bivalents ranged from 10-11 and 0-1 with 10.4 and 0.56 per cell respectively. At anaphase-I and II equal separation of chromosomes to the poles was observed. Pollen fertility was 98.64%.

(ii) 0.4%:

Bivalents was the only association at metaphase-I. At anaphase-I and II equal separation of chromosomes was observed (Table-155). Pollen fertility percentage was 98.23.

(iii) 0.6%:

At metaphase-I, quadrivalents and trivalents ranged from 0-1 and 0-1 with 0.02 and 0.03 per cell respectively. Ring bivalents ranged from 8-11 with 7.55 per cell and rod bivalents ranged from 0-3 with 2.97 per cell. At the same stage univalents ranged from 0-2 with 0.35 per cell. At anaphase-I, delayed separation and laggards were observed in 2.0 and 2.0 per cent cells respectively. At anaphase-I bridge was also noticed in 2.0 per cent cells. At anaphase-II, equal separation of chromosomes was observed in all the cells studied and at sporad stage only tetrad formation was noticed resulting in 97.52 per cent pollen fertility.

(iv) 0.8%:

At metaphase-I, quadrivalents and trivalents (Plate-26; Fig. 4) ranged from 0-1 and 0-1 with 0.04 and 0.03 per cell respectively. Gradual increase in the frequency of rod bivalents and decrease in ring bivalents was recorded as ring bivalents ranged from 6-11 with 7.50 per cell and rod bivalents ranged from 0-5 with 3.16 per cell (Table-155). At the same stage, univalents (Plate-26; Fig. 5) ranged from 0-5 with 0.25 per cell. In some cells arrangement of bivalents into two groups at metaphase-I was recorded. At anaphase-I and II laggards were observed in 2.10 and 1.05 per cent cells respectively. At sporad stage, other than tetrads, one to micronuclei (Plate-26; Fig. 11) were observed. Pollen fertility was 96.81%. At anaphase-I double chromatid bridge was recorded in 2.10% cells (Plate-26; Fig. 8).

8 hours treatment:(i) 0.2%:

No meiotic abnormality was observed. Pollen fertility was 92.55 per cent.

(ii) 0.4%:

At metaphase-I, ring bivalents ranged from 8-11 with 9.97 per cell and rod bivalents ranged from 0-3 with 1.0 per cell. A range of 0-2 univalents with 0.05 per cell was also recorded at metaphase-I. At anaphase-I and II, equal separation of chromosomes to the poles was observed resulting in 98.0 per cent pollen fertility.

(iii) 0.6%:

At metaphase-I quadrivalents and trivalents ranged from 0-1 and 0-1 with 0.01 and 0.01 per cell respectively. Ring bivalents ranged from 8-11 with 7.92 per cell and

Table - 152

Germination of EMS treated seeds of Atylosia platycarpa
(JM 2873) No. of seeds treated in each case was 20.

| Concen- tration (%) | Duration of treat- ment (hours) | Germination of seeds in petridish (%) | Emergence of plumule in field (%) | Survival to maturity (%) |
|---------------------------|--|--|--|--------------------------------|
| Control | - | 100 | 95.0 | 100 |
| 0.2 | 4 | 95.0 | 90.0 | 85.0 |
| " | 8 | 90.0 | 85.0 | 80.0 |
| 0.4 | 4 | 85.0 | 80.0 | 75.0 |
| " | 8 | 80.0 | 70.0 | 60.0 |
| 0.6 | 4 | 75.0 | 65.0 | 55.0 |
| " | 8 | 70.0 | 60.0 | 50.0 |
| 0.8 | 4 | 65.0 | 55.0 | 45.0 |
| " | 8 | 60.0 | 40.0 | 30.0 |
| 1.0 | 4 | 30.0 | NIL | - |
| " | 8 | 20.0 | NIL | - |

Morphological observations in control, M₁ and M₂ plants of *Atylosia platycarpa*

| Characters | Control | Gene- ration | 4 hours treatment | | | | 8 hours treatment | | | |
|--------------------------------|---------|-----------------|-------------------|---------|---------|---------|-------------------|---------|---------|---------|
| | | | 0.2% | 0.4% | 0.6% | 0.8% | 0.2% | 0.4% | 0.6% | 0.8% |
| Plant spread (cm) | 36 | M ₁ | 35.5 | 34.2 | 36.4 | 35.2 | 37.1 | 30.4 | 32.3 | 31.4 |
| | 38 | M ₂ | 38.4 | 37.1 | 34.4 | 35.1 | 32.3 | 33.5 | 38.9 | 34.5 |
| No. of primary branches | 5 | M ₁ | 4.2 | 6.7 | 5.2 | 4.1 | 6.1 | 5.7 | 5.4 | 5.3 |
| | 6 | M ₂ | 6.3 | 5.6 | 7.5 | 5.0 | 6.3 | 6.0 | 5.7 | 5.8 |
| No. of secondary branches | 7 | M ₁ | 6.1 | 7.1 | 7.5 | 5.1 | 7.1 | 8.2 | 7.5 | 6.5 |
| | 8 | M ₂ | 8.2 | 8.0 | 7.6 | 5.3 | 7.3 | 8.3 | 7.9 | 7.1 |
| Central leaflet (L x B) cm. | 4.5x4.0 | M ₁ | 4.3x4.0 | 4.1x4.2 | 4.0x3.8 | 4.9x4.0 | 4.2x3.9 | 4.0x4.0 | 4.2x4.1 | 4.1x4.0 |
| | 4.3x4.1 | M ₂ | 4.7x4.0 | 4.6x4.1 | 4.7x3.9 | 5.0x4.6 | 4.5x4.1 | 4.2x4.1 | 4.5x4.3 | 4.4x4.1 |
| Days to flowering | 58 | M ₁ | 59 | 60 | 62 | 64 | 60 | 61 | 63 | 65 |
| | 56 | M ₂ | 60 | 61 | 61 | 62 | 61 | 60 | 62 | 62 |
| Days to maturity | 126 | M ₁ | 126 | 127 | 130 | 134 | 128 | 129 | 130 | 132 |
| | 128 | M ₂ | 128 | 129 | 131 | 138 | 130 | 131 | 133 | 136 |
| Pods per plant | 32 | M ₁ | 28.1 | 22.4 | 16.3 | 7.5 | 30.1 | 30.2 | 14.4 | 9.5 |
| | 35 | M ₂ | 30.2 | 32.6 | 34.4 | 22.5 | 34.1 | 38.3 | 26.6 | 28.3 |
| Seeds per pod | 2.6 | M ₁ | 2.3 | 1.7 | 1.5 | 1.1 | 1.3 | 1.7 | 1.5 | 1.0 |
| | 2.5 | M ₂ | 2.5 | 2.2 | 2.4 | 1.5 | 1.9 | 2.2 | 2.5 | 2.1 |

* In each generation 5 plants were studied

341

Table - 154

Mitotic observations in M_1 seeds of Alysiola platycarpa (Per cent in parentheses) .

| | | | METAPHASE | | | | ANAPHASE | | |
|---------------------------|-------------------------|----------------------------|-----------------------|-----------------------------|----------------|---------------|----------------------------|---------------------------|----------------------|
| Concent- ration (%) | Durat- ion (hrs.) | No. of cells studied | Uneffect- ed cells | Chromo- some breakage | Stick- ness | Clump- ing | No. of cells studied | Normal separa- tion | Bridge + fragment |
| Control | - | 30 | 30 (100) | - | - | - | 25 | 25 (100) | - |
| 0.2 | 4 | 60 | 60 (100) | - | - | - | 30 | 30 (100) | - |
| " | 8 | 35 | 35 (100) | - | - | - | 25 | 25 (100) | - |
| 0.4 | 4 | 50 | 47 (94.0) | - | 1 (2.0) | 2 (4.0) | 30 | 30 (100) | - |
| " | 8 | 50 | 41 (82.0) | - | 2 (4.0) | 3 (6.0) | 25 | 25 (100) | - |
| 0.6 | 4 | 50 | 45 (90.0) | - | 3 (6.0) | 2 (4.0) | 40 | 40 (100) | - |
| " | 8 | 25 | 20 (80.0) | 1 (4.0) | 2 (8.0) | 2 (8.0) | 25 | 24 (96.0) | 1 (4.0) |
| 0.8 | 4 | 50 | 41 (82.0) | 1 (2.0) | 4 (8.0) | 4 (8.0) | 50 | 49 (98.0) | 1 (2.0) |
| " | 8 | 50 | 37 (74.0) | 2 (4.0) | 5 (10.0) | 6 (12.0) | 50 | 48 (96.0) | 2 (4.0) |
| 1.0 | 4 | 50 | 16 (32.0) | 3 (6.0) | 16 (32.0) | 15 (30.0) | 50 | 48 (96.0) | 2 (4.0) |
| " | 8 | 50 | 15 (30.0) | 4 (8.0) | 15 (30.0) | 16 (32.0) | 50 | 46 (92.0) | 3 (6.0) |

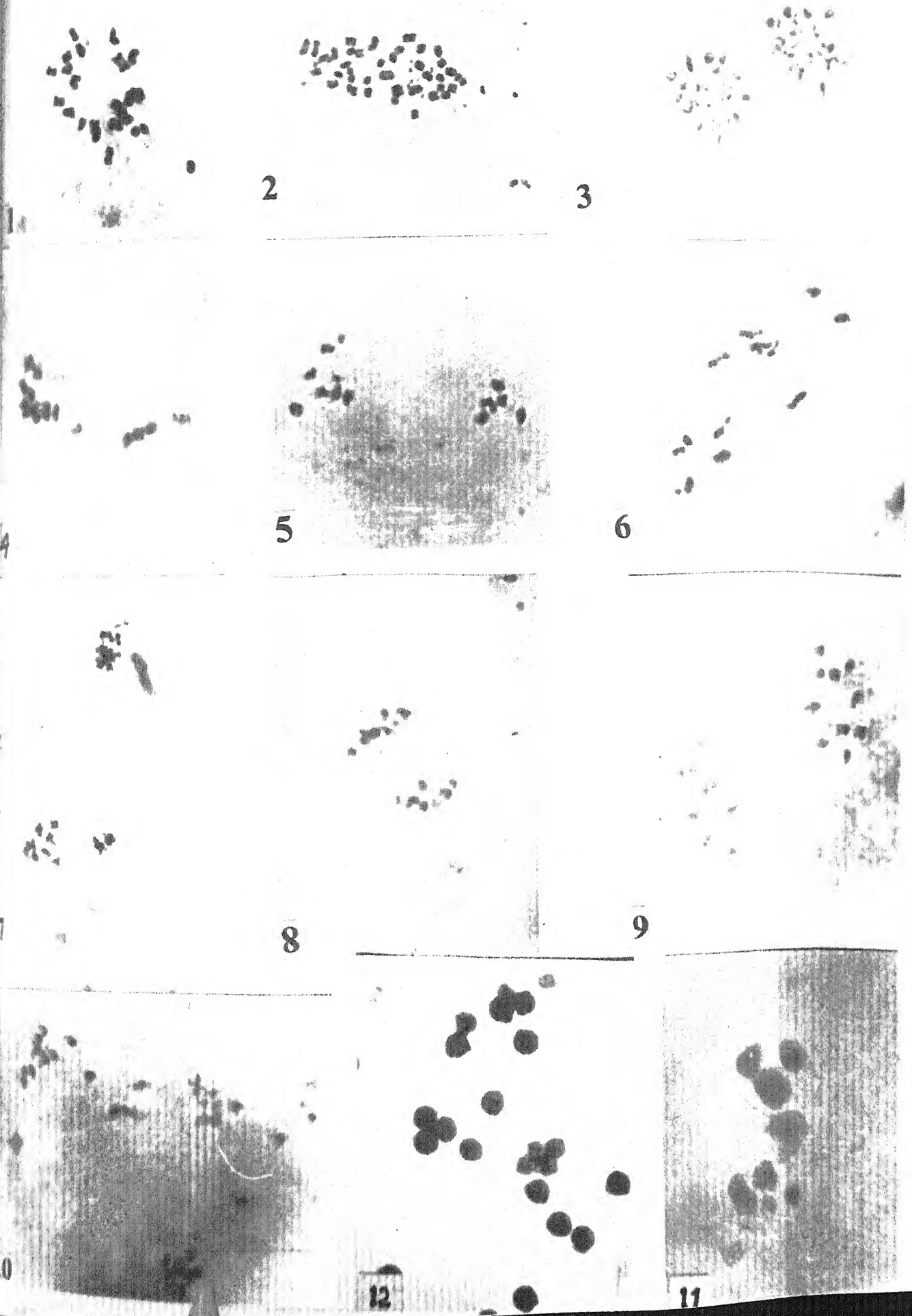
Table - 155

Meiotic observations in M_1 plants of Atylosia platycarpa (JM 2873) No. of plants studied in each case were 5.

| Concen- tration (%) | dura- tion (hrs.) | No. of cells studi- ed | Chromosomal associations at | | | | Anaphase - I | | | | Anap. II | | |
|---------------------------|-------------------------|---------------------------------|-----------------------------|---------------|------------------|---------------|---------------------------------|--------------------------|--------------------|--------------------|---------------------------------|--------------------|---------------------------------|
| | | | Metaphase - I | | | | No. of cells studi- ed | Delay- ed sep. (%) | Lag- gs. (%) | Brid- ge (%) | No. of cells studi- ed | Lag- gs. (%) | pollen ferti- lity (%) |
| | | | IV | III | Ring II | Rad II | | | | | | | |
| Control | - | 85 | - | - | 10-11 (10.82) | 0-1 (0.17) | - | - | - | - | 80 | - | 99.83 |
| 0.2 | 4 | 82 | - | - | 10-11 (10.4) | 0-1 (0.56) | - | - | - | - | 65 | - | 98.64 |
| " | 8 | 50 | - | - | 10-11 (10.35) | 0-1 (0.65) | - | - | - | - | 58 | - | 98.55 |
| 0.4 | 4 | 80 | - | - | 8-11 (10.00) | 0-3 (0.97) | - | - | - | - | 56 | - | 98.23 |
| " | 8 | 72 | - | - | 8-11 (9.97) | 0-3 (1.00) | 0-2 (0.05) | - | - | - | 61 | - | 98.00 |
| 0.6 | 4 | 67 | 0-1 (0.02) | 0-1 (0.03) | 8-11 (7.55) | 0-3 (2.97) | 0-2 (0.35) | 2.0 | 2.0 | 2.0 | 60 | - | 97.52 |
| " | 8 | 62 | 0-1 (0.01) | 0-1 (0.01) | 8-11 (7.92) | 0-3 (2.85) | 0-2 (0.33) | 2.0 | 4.0 | - | 50 | 2.0 | 95.11 |
| 0.8 | 4 | 120 | 0-1 (0.04) | 0-1 (0.03) | 6-11 (7.50) | 0-5 (3.16) | 0-5 (0.25) | 1.05 | 2.10 | 2.10 | 95 | 1.05 | 96.81 |
| " | 8 | 75 | 0-1 (0.02) | 0-1 (0.04) | 6-11 (7.69) | 0-5 (3.55) | 0-5 (0.36) | 2.00 | 4.00 | - | 50 | 2.00 | 93.00 |

(Mean values in parentheses)

PLATE - 26



rod bivalents ranged from 0-3 with 2.85 per cell. Univalents, at metaphase-I ranged from 0-2 with 0.33 per cell. At anaphase-I and II, laggards were observed in 4.0 and 2.0 per cent cells respectively. At sporad stage tetrads were of usual occurrence. Occasionally a micronuclei was noticed. Pollen fertility percentage was 95.11.

(iv) 0.8%:

At metaphase-I, quadrivalents ranged from 0-1 with 0.02 per cell and trivalents ranged from 0-1 with 0.04 per cell. Ring and rod bivalents (Plate-26; Fig. 7) ranged from 6-11 and 0-5 with 7.69 and 3.55 per cell respectively. Univalents ranged from 0-5 with 0.36 per cell. At anaphase-I and II, laggards (Plate-26; Fig. 10) were observed in 4.0 and 2.0 per cent cells respectively. At sporad stage other than tetrads, micronuclei were also recorded. Pollen fertility (Plate-26; Fig. 12) was 93.0 per cent.

Effects of EMS on seed germination and plant survival in *Atylosia lineata* (JM 2639).

Observations on seed germination in petridishes, emergence of plumules in field and survival to maturity in 4 and 8 hours treatments at different concentrations of EMS are as follows:

4 hours treatment:

After treatment with the lowest concentration (0.2%) of EMS solution, a slight decline in the percentage of seed germination, plumule emergence and plant survival to maturity was recorded in comparison to control. At higher concentration (0.4%), further decrease in per cent

seed germination, plumule emergence and survival to maturity was registered (Table-155). In the treatment with 0.5% EMS solution, per cent seed germination, plumule emergence and plant survival were 50.0, 58.0 and 71.4 respectively. When 0.8% concentration used for 4 hours only 10.0 per cent seeds germinated, while plumules could not emerge after this treatment. In the treatment with the highest concentration of EMS solution (1.0%) no seed germination was recorded, hence no seedling could be raised.

8 hours treatment:

At 0.2% concentration, slight decrease in per cent seed germination, plumule emergence and plant survival to maturity was noticed in comparison to control. 0.4% EMS solution when used for 8 hours, seed germination, plumule emergence and plant survival were 76.0, 60.0 and 79.0 per cent respectively. At 0.6% concentration, further reduction in the percentage of seed germination, plumule emergence and plant survival was recorded (Table-155). At 0.8% concentration, only 8 per cent seed germination was noticed but no plumule could emerge after the treatment. When the highest concentration of EMS (1.0%) was used no seed could germinate (Table-155).

Morphological observations in EMS treated plants of *A. lineata* (JM 2639).

Morphological observations in control, M_1 and M_2 plants of *A. lineata* are summarised in Table-156. The details are as follows.

4 hours treatment:0.2%:

M_1 plants showed 92.4 cm average plant height and 3.8 and 6.2 average primary and secondary branches respectively. Plants showed 4.5 cm average length and 2.1 cm average breadth of central leaflet. Days to 50% flowering and maturity were 128 and 196 respectively. On an average, number of pods per plant was 46.0 and seeds per pod 1.8 respectively.

M_2 plants showed 99.1 cm average height, 4.2 primary branches and 6.5 secondary branches. The average length and breadth of the central leaflets were 4.6 and 2.1 cm respectively. Days to 50% flowering and maturity were nearer to those of control plants. On an average, number of pods per plant was 50.1 and seeds per pod 1.9.

0.4%:

On an average, the M_1 plants showed 90.3 cm height, 3.6 primary branches and 6.1 secondary branches. Central leaflet length was 4.6 and breadth 2.2 cm. Days to 50% flowering and maturity were 130 and 196 respectively. Average number of pods per plant was 33.3 and seeds per pod 1.3.

M_2 plants had average 98.5 cm height, 3.6 primary and 6.1 secondary branches. The average length and breadth of central leaflet were 4.7 cm and 2.2 cm respectively. Days to 50% flowering and maturity were nearer to those of control plants. On an average, number of pods per plant was 47.2 and seeds per pod 1.8.

0.6%:

M_1 plants showed, average plant height of 81.5 cm. Number of primary and secondary branches were 3.6 and 6.1 respectively. The average length and breadth of central leaflet were 4.3 and 2.0 cm respectively. Days to 50% flowering and maturity were 133 and 196 respectively. While in control plants these were 130 and 197 respectively. On an average, number of pods per plant was 20.1 and seeds per pod 0.9.

M_2 plants had an average height of 97.3 cm, 4.1 primary branches and 6.1 secondary branches. Plants showed 4.8 cm average leaf let length and 2.4 cm breadth. Days to 50% flowering and maturity were nearer to those of control plants on an average, number of pods per plant was 41.5 and seeds per pod. 1.7.

8 hours treatment:0.2%:

M_1 plants had 93.1 cm average height, 3.5 primary branches and 6.7 secondary branches. Plants showed 4.1 cm average length of central leaflet and 2.0 cm breadth. Days to 50% flowering and maturity were 130 and 195. On an average, number of pods per plant was 41.2 and seeds per pod 1.7.

In M_2 plants, average 99.5 cm height, 4.5 primary branches and 6.8 secondary branches were recorded. The average length of central leaflet was 4.7 cm and breadth 2.1. Days to 50% flowering and maturity were nearer to those of control plants. Pods per plant was 46.1 and seeds per pod. 1.8.

0.4 %:

M_1 plants showed 91.7 cm average height, 3.4 primary branches and 6, 3 secondary branches. Plants showed 4.4 cm average length and 2.2 cm breadth of central leaflets. Days to 50% flowering and maturity were 129 respectively. Pods per plant was 41.5 and seeds per pod 1.2.

M_2 plants showed 93.6 cm average height, 4.4 primary branches and 6.4 secondary branches. Length of central leaflet was 4.5 cm and breadth 2.2 cm. Days to 50% flowering and maturity were nearer to those of control plants. Number of pods per plant was 45.2 and seeds per pod 1.5.

0.6 %:

Average height of M_1 plant was 83.6 cm and 3.1 primary branches and 6.1 secondary branches. Average central leaf length and breadth were 4.0 and 2.0 cm respectively. In M_1 plants, other than trifoliate leaves, quadrifoliate and pentafoolate leaves were (Plate-27; Fig.1) also noticed. Days to 50% flowering and maturity were 134 and 200 as against 130 and 197 in control plants. Number of pods per plant was 18.5 and seeds per pod 1.0.

M_2 plants showed 90.2 cm average height, 4, 2 primary branches and 6.3 secondary branches. Central leaflet length was 4.1 cm and breadth 2.0 cm. Days to 50% flowering and maturity were nearer to those of control plants. Number of pods per plant was 40.1 and seeds per pod 1.6.

Corresponding decrease in the plant height, average number of primary and secondary branches, number

of pods per plant and seeds per pod was noticed with increase in concentration and duration of treatment.

Cytology (M_1):

Mitosis:

Observations made in the root tip cells of M_1 seeds revealed chromosome stickiness, clumping and breakage (Plate-27). Chromosomal abnormalities detectable during mitotic divisions are summarised in Table-157. Details are as follows:

4 hours treatment:

At 0.2% concentration of EMS, no cytological abnormality was noticed. At the next higher concentration (0.4%) chromosome stickiness, clumping and breakage was observed in 6.6, 3.3 and 3.3 of cells respectively. The treatment of 0.6% concentration of EMS solution, further increased the percentage of cells showing chromosome stickiness, clumping and breakage (Plate-27; Fig. 2) (Table-157). In the treatment with the highest concentration (0.8%), 32.0 per cent cells were scored showing chromosome breakage. At anaphase, bridge with fragments (Plate-27; Fig. 5) and bridge without fragment were observed in 4.0 and 8.2 per cent cells respectively. In 4.0 per cent cells laggards (Plate-27; Fig. 7), were observed.

8 hours treatment:

Normal mitosis was observed after the treatment with 0.2% EMS solution. Treatment with 0.4% EMS solution revealed stickiness, clumping and chromosome breakage 8.0, 4.0 and 4.0 per cent cells respectively. At anaphase bridge was observed in 4.0 per cent cells. At anaphase

single, double and multiple bridges were observed (Plate-27; Fig. 6) was observed in 28.12 per cent cells. At anaphase, bridge with fragment and without fragment was observed in 3.57 and 7.14 per cent cells respectively. The highest concentration (0.8%) resulted in chromosome stickiness, clumping and breakage in 20.0, 16.0 and 28.12 per cent cells respectively. At anaphase bridge with and without fragment were recorded in 8.0 and 12.0 per cent cells respectively.

Thus a corresponding increase in chromosome stickiness, clumping and breakage was observed with increase in concentration and duration of treatment (Table-157).

Meiosis (M_1 plants):

Meiotic studies in M_1 plants revealed trivalents, bivalents, and univalents at metaphase-I (Plate-27). Observations on chromosomal associations (Table-158) at each concentration and duration of treatment are as follows:

4 hours treatment:

0.2 % :

The lowest concentration (0.2%) seems to have no effects on the meiotic chromosomes as is evident by the regular formation of bivalents. However, a range of 0-4 univalents with 0.43 per cell was observed. At anaphase-I and II equal separation of chromosomes to the poles, alongwith regular tetrad formation and higher pollen fertility (94.61%) was noticed.

0.4 % :

Meiotic abnormalities were seen with the increase in the concentration. At metaphase-I trivalents ranged from 0-2 with 0.04 per cell. Ring and rod bivalents ranged from 2-11 and 0-9 with 4.46 and 6.00 per cell respectively. Univalents (Plate-27; Fig. 8) ranged from 0-14 with 0.85 per cell. At anaphase-I delayed separation of one bivalent was observed in 2.5% cells. Laggards at anaphase-I and II were observed in 5.0 and 2.0 cells respectively. At sporad stage, other than tetrads, micronuclei and polyads were also noticed. Pollen fertility was 82.52%.

0.6 % :

Frequency of trivalents and univalents were more (0.17 and 2.17) in contrast to those observed at 0.4% concentration of EMS. At anaphase-I, delayed separation of bivalents was noticed in 5.0% of cells. Laggards at anaphase-I (Plate-27; Fig. 11) and II were observed in 5.0 and 2.5 per cent cells respectively. Occasionally, at anaphase-II, grouping of chromosomes in more than 2 groups were recorded. At sporad stage, other than regular tetrads, polyads and micronuclei were also seen. Pollen fertility was 75.68 per cent (Table-138).

8 hours treatment:0.2 % :

The lowest concentration (0.2%) of EMS used for longer duration (8 hours) appeared to have visible effects on meiotic chromosomes. A range of 0-4 univalents with 0.15 per cell was noticed. Pollen fertility was 92.53 per cent.

Table-155

Germination of EMS treated seeds of Alysicarpus lineata
(JM 2639). No. of seeds treated in each case was 50

| Concen- tration of EMS (%) | Dura- tion of treat- ment (hours) | Germina- tion in petridish (%) | Emergence of plumules in field (%) | Survival to natu- rity (%) |
|-------------------------------------|--|---|---|----------------------------------|
| Control | - | 97.0 | 96.0 | 96.8 |
| 0.2 | 4 | 90.0 | 86.0 | 92.3 |
| " | 8 | 86.0 | 82.0 | 91.3 |
| 0.4 | 4 | 80.0 | 62.0 | 80.0 |
| " | 8 | 76.0 | 60.0 | 79.1 |
| 0.6 | 4 | 50.0 | 58.0 | 71.4 |
| " | 8 | 40.0 | 55.0 | 69.6 |
| 0.8 | 4 | 10.0 | NIL | - |
| " | 8 | 8.0 | NIL | - |
| 1.0 | 4 | NIL | - | - |
| " | 8 | NIL | - | - |

Morphological observations in control, M₁ and M₂ plants of *Alysicarpus lineatus* (JN2639)

| Characters | Control | Gene- ration * | 4 hours treatment | | | 8 hours treatment | | |
|----------------------------|---------|----------------------|-------------------|---------|---------|-------------------|---------|---------|
| | | | 0.2% | 0.4% | 0.6% | 0.2% | 0.4% | 0.6% |
| Plant height (cm) | 98.3 | M ₁ | 92.4 | 90.3 | 81.5 | 93.1 | 91.7 | 83.6 |
| | 95.2 | M ₂ | 99.1 | 98.5 | 97.3 | 94.5 | 93.6 | 90.2 |
| No. of primary branches | 3.7 | M ₁ | 3.8 | 3.6 | 3.9 | 3.5 | 3.4 | 3.1 |
| | 3.5 | M ₂ | 4.2 | 4.0 | 4.1 | 4.5 | 4.4 | 4.2 |
| No. of secondary branches | 6.4 | M ₁ | 6.2 | 6.1 | 6.0 | 6.7 | 6.3 | 6.1 |
| | 6.3 | M ₂ | 6.5 | 6.7 | 6.1 | 6.8 | 6.4 | 6.3 |
| Central leaflet (L x B) cm | 4.8x2.0 | M ₁ | 4.5x2.1 | 4.6x2.2 | 4.3x2.0 | 4.1x2.0 | 4.4x2.2 | 4.0x2.0 |
| | 4.6x2.1 | M ₂ | 4.6x2.1 | 4.7x2.3 | 4.8x2.4 | 4.7x2.1 | 4.5x2.3 | 4.1x2.0 |
| Days to flowering | 130 | M ₁ | 128 | 130 | 133 | 130 | 129 | 134 |
| | 126 | M ₂ | 125 | 128 | 130 | 127 | 130 | 129 |
| Days to maturity | 197 | M ₁ | 196 | 196 | 199 | 195 | 197 | 200 |
| | 195 | M ₂ | 195 | 194 | 196 | 196 | 195 | 195 |
| Pods per plant | 45.3 | M ₁ | 46.0 | 33.3 | 20.1 | 41.2 | 31.5 | 18.5 |
| | 49.8 | M ₂ | 50.1 | 47.2 | 41.5 | 46.1 | 45.2 | 40.1 |
| Seeds per pod | 1.91 | M ₁ | 1.8 | 1.3 | 0.4 | 1.7 | 1.2 | 1.0 |
| | 1.70 | M ₂ | 1.9 | 1.8 | 1.7 | 1.8 | 1.5 | 1.6 |

* In each generation number of plants studied were 10

Table - 157

Mitotic observations in M_1 seeds of Alysis lineata (JM 2639) •

| | | M E T A P H A S E | | | | A N A P H A S E | | | | |
|---------------------------|-------------------------|----------------------------|-----------------------|-----------------------------|-----------------|-----------------|----------------------------|---------------------------|----------------------|-------------|
| Concent- ration (%) | Durat- ion (hrs.) | No. of cells studied | uneffect- ed cells | Chromo- some breakage | Sticki- ness | Clump- ing | No. of cells studied | Normal separa- tion | Bridge + fragment | lags. |
| Control | - | 25 | 25 (100) | - | - | - | 25 | 25 (100) | - | - |
| 0.2 | 4 | 25 | 25 (100) | - | - | - | 25 | 25 (100) | - | - |
| 0.2 | 8 | 30 | 30 (100) | - | - | - | 30 | 30 (100) | - | - |
| 0.4 | 4 | 30 | 26 (86.6) | 1 (3.33) | 2 (6.66) | 1 (3.33) | 30 | 29 (96.60) | 1 (3.33) | - |
| 0.4 | 8 | 25 | 21 (84.0) | 1 (4.00) | 2 (8.00) | 1 (4.00) | 25 | 24 (96.0) | 1 (4.0) | - |
| 0.6 | 4 | 40 | 18 (45.0) | 10 (25.0) | 6 (15.0) | 6 (15.0) | 30 | 25 (83.3) | 3 (10.0) | 1 (3.33) |
| 0.6 | 8 | 32 | 13 (40.6) | 9 (28.12) | 5 (15.65) | 5 (15.65) | 28 | 24 (85.71) | 2 (7.14) | 1 (3.57) |
| 0.8 | 4 | 25 | 9 (36.0) | 8 (32.0) | 4 (16.0) | 4 (16.0) | 25 | 22 (88.0) | 2 (8.0) | 1 (4.0) |
| 0.8 | 8 | 25 | 9 (36.0) | 7 (28.12) | 5 (20.0) | 4 (16.0) | 25 | 20 (80.0) | 3 (12.0) | 2 (8.0) |

(figures in parentheses are per cent)

Table - 158

Meiotic observations in M_1 plants of Atylosia lineata (JM 2639). No. of plants studied in each case were 5.

Case were 3:

| Concen- tration (%) | Dura- tion (hrs.) | No. of cells studi- ed | Chromosomal associations | | | | | Anaphase - I | | Anaphase - II | | Pollen ferti- lity (%) |
|---------------------------|-------------------------|---------------------------------|--------------------------|----------------|----------------|----------------|---------------------------------|-----------------------------|--------------------|----------------------------|---------------|---------------------------------|
| | | | at Metaphase - I | | | I | No. of cells studi- ed | Dele- yed sep. (%) | Lag- gs. (%) | No. of cells studied | Laggs. (%) | |
| | | | III | Ring II | Red II | | | | | | | |
| Control | - | 75 | - | 9-11 (10.6) | 0-2 (0.4) | - | 50 | - | - | 80 | - | 99.37 |
| 0.2 | 4 | 72 | - | 5-11 (8.47) | 0-6 (2.40) | 0-4 (0.43) | 50 | - | - | 60 | - | 94.61 |
| " | 8 | 64 | | 5-11 (8.32) | 0-6 (2.60) | 0-4 (0.15) | 50 | - | - | 50 | - | 92.55 |
| 0.4 | 4 | 47 | 0-2 (0.04) | 2-11 (4.46) | 0-9 (6.00) | 0-14 (0.85) | 40 | 2.5 | 5.0 | 50 | 2.00 | 82.52 |
| " | 8 | 43 | 0-2 (0.04) | 3-11 (4.43) | 0-8 (6.10) | 0-14 (0.79) | 50 | 2.00 | 4.0 | 50 | 2.00 | 80.00 |
| 0.6 | 4 | 35 | 0-3 (0.17) | 0-11 (2.05) | 0-11 (6.77) | 0-14 (2.17) | 40 | 5.0 | 5.0 | 41 | 2.50 | 75.68 |
| " | 8 | 37 | 0-3 (0.16) | 0-11 (2.26) | 0-11 (3.55) | 0-14 (2.08) | 50 | 6.0 | 8.0 | 40 | 5.00 | 72.51 |

(Mean values in parentheses)

33
37
57

PLATE - 27 (Effect of EMS on A. lineata)

Fig. 1-7 : Mitosis, 8-14; Meiosis.

Fig. 1. Trifoliate, quadrifoliate, and pentafoliate leaves of A. lineata (0.6%)

Fig. 2. Chromosome breakage at prophase (0.4%) (X 1500)

Fig. 3. Chromosomes fragmentation (0.6%) Metaphase (X 1500)

Fig. 4. Chromosome fragmentation (0.8%) at Metaphase (X 1500)

Fig. 5. Bridge + fragment at Anaphase (X 1500)

Fig. 6. Multiple bridge + fragment (X 1500)

Fig. 7. Laggards at somatic telophase (X 1500)

Fig. 8 . 6 II's + 10 I's at Metaphase-I, showing two dividing univalents (0.4%) (X 1500)

Fig. 9. 2 III's + 8 II's at Metaphase-I (0.6%) (X 1500)

Fig. 10. 2 III's + 1 II + 14 I's at Metaphase-I (0.6%) (X 1500)

Fig. 11. Laggards at Anaphase-I (0.6%) (X 1500)

Fig. 12. Delayed separation of one bivalent at Anaphase-I (0.6%) (X 1500)

Fig. 13. 8 unequal groups of chromatids at Anaphase-II (0.6%) (X 1500)

Fig. 14. Hexad (X 600)

PLATE - 27



1

2

3

4

5

6

7

8

9

11

10

12

13

14

0.4 % :

At metaphase-I, trivalents (Plate-27; Fig. 9) ranged from 0-2 with 0.04 per cell. Ring bivalents ranged from 3-11 with 4.43 per cell while rod bivalents ranged from 0-8 with 6.10 per cell. Univalents, at metaphase-I ranged from 0-14 with 0.79 per cell. At anaphase-I and II, laggards were noticed in 4.0 and 2.0 per cent cell respectively. At sporad stage tetrads, polyads and micronuclei were observed. Pollen fertility was 80.0%.

0.6 % :

At metaphase-I, trivalents ranged from 0-3 with 0.16 per cell. Ring and rod bivalents ranged from 0-11 and 0-11 with 2.39 and 7.55 per cell respectively. Univalents (Plate-10; Fig. 10) at metaphase-I ranged from 0-14 with 1.02 per cell. At anaphase-I delayed separation of one bivalent (Plate-27; Fig. 12) was recorded in 6.0 per cent cells. Laggards at anaphase-I and II were noticed in 8.0 per cent and 5.0 per cent cells. In frequency at anaphase-II chromatids in more than 4 groups were seen (Plate-27; Fig. 13). At sporad stage other than tetrads, micronuclei as well as polyads (Plate-27; Fig. 14) were formed. Pollen fertility percentage was 72.51 (Table-158).

Effects of EMS on seed germination and plant survival in
Atylosia volubilis.

There was a corresponding reduction in the percentage of seed germination and plant survival with the increase in concentration and duration of treatment (Table-159). Observations on seed germination in petridishes, emergence of plumules in field and survival to maturity in

4 and 8 hours treatments at different concentrations are as follows:

4 hours treatment:

The treatment with the lowest concentration (0.2%) of EMS for 4 hours resulted in slight decrease in the percentage of seed germination, plumule emergence and plant survival in comparison to the control (Table-159). At 0.4 per cent concentration, percentage seed germination, plumule emergence and survival to maturity were 80.0, 81.25 and 92.30 respectively. With further increase in concentrations (0.6% and 0.8%) of EMS solutions, gradual reduction in the percentage of seed germination, plumule emergence and survival to maturity was registered (Table-159). At the highest concentration of 1.0 per cent EMS solution, no seed could germinate.

8 hour treatment:

Increase in the duration of the treatment from 4 to 8 hours could not appreciably change the percentage of seed germination, plumule emergence and survival to maturity. At the higher concentration (0.4%) of EMS solution, there was a decrease in the percentage of seed germination, plumule emergence and plant survival as compared to 4 hours treatment. Further decrease in percentage germination of seed, plumule emergence and plant survival was noticed in the treatment with 0.6% such decrease was significant (Table-159).

Morphological observations in EMS treated plants of *Atylosia volubilis*.

Morphological observations in control, M_1 and M_2 plants of *A. volubilis* are summarised in Table-160. Their details are as follows:

4 hours treatment:0.2 per cent :

M_1 plants showed 75.1 cm average plant spread. Number of primary and secondary branches 0.5 and 10.5 respectively. Plants showed 4.3 cm average length of central leaflet and 4.1 cm average breadth of leaves. Days to 50% flowering and maturity were 201 and 275 as against 203 and 277 in control plants. On an average pods per plant was 32.1 and seeds per pod 2.5.

In M_2 plants, average plant spread was 76.2 cm. Number of primary and secondary branches were 6.6 and 11.2 respectively. Average length of central leaflet was 4.5 cm and breadth 4.2 cm. Days to 50% flowering and maturity were nearer to those of control plants. Average number of pods per plant was 34.5 and seeds per pod was 2.4.

0.4 per cent:

M_1 plants showed 70.0 cm average plant spread. On an average, number of primary and secondary branches were 6.4 and 9.8 respectively. Plants showed 4.2 cm average length of central leaflet and 4.0 cm breadth. Days to 50% flowering and maturity were 203 and 275 respectively. Average number of pods per plant was 28.1 and seeds per pod, 2.1.

Average plant spread of M_2 was 72.5 cm. Number of primary and secondary branches were 6.8 and 10.5 respectively. Average length of central leaflet was 4.3 cm and breadth 4.1 cm. Days to 50% flowering and maturity were nearer to those of control plants. On an average, number of pods per plant was 30.5 and seeds per pod 2.2.

0.6 per cent:

Average plant spread of M_1 was 46.2 cm as against 76.0 cm in control. Decrease in the number of primary as well as secondary branches and also in leaflet sizes was noticed in M_1 , in comparison to control. Days to 50% flowering and maturity were 205 and 279 respectively. On an average the number of pods per plant was 11.2 and seeds per pod 1.1.

M_2 plants showed 70.0 cm average plant spread. M_2 s likewise M_1 s showed reduction of number of primary as well as secondary branches and also central leaflet size in comparison to control. Days to 50% flowering and maturity were nearer to those of control plants. On an average, number of pods per plant was 25.6 and seeds per pod 2.0.

8 hours treatment:0.2 per cent:

M_1 plants showed 69.0 cm average plant spread. Number of primary and secondary branches were 5.8 and 9.2 respectively central leaf let was 4.2 cm in length and 4.1 cm in breadth. Days to 50% flowering and maturity were 20 x and 277 respectively. On an average number of pods per plant was 30.4 and seeds per pod 2.6.

Average plant spread of M_2 s was 71.3 cm. Number of primary and secondary branches were 6.0 and 11.5 respectively. Average length of central leaflet was 4.3 cm and breadth 4.1 cm. Days to 50% flowering and maturity were nearer to those of control plants. Number of pods per plant was 32.5 and seeds per pod 2.7.

0.4 per cent:

Average plant spread in M_1 s was 70.0 cm. Number of primary and secondary branches were 4.7 and 9.0 respectively. In comparison to control, there was decrease in the central leaflet size. Other than trifoliate, unifoliate and bifoliate leaves were also noticed. Days to 50% flowering and maturity were 203 and 278. Number of pods per plant was 18.2 and seeds per pod 2.0.

Average plant spread was 72.5 cm in M_2 s. Number of primary and secondary branches were 6.5 and 9.7 respectively. Further decrease in central leaflet size was noticed with increased concentration of EMS. Days to 50% flowering and maturity were nearer to those of control plants. The number of pods per plant was 26.5 and seeds per pod 2.1.

0.6 per cent:

Average plant spread was 48.5 cm in M_1 s. Number of primary and secondary branches were 3.8 and 8.0 respectively. Considerable decrease in central leaflet size was noticed and other than trifoliate leaves, unifoliate and bifoliate leaves were also noticed (Plate-28; Fig. 1). Days to 50% flowering and maturity were 204 and 279 respectively.

Average plant spread in M_2 plants was 70.5 cm. Number of primary and secondary branches were 5.7 and 8.1 respectively. A decrease in central leaflet size recorded similar to F_1 . Days to 50% flowering and maturity were nearer to those of control plants. On an average number of pods per plant was 25.5 and seeds per pod 2.0.

Cytology (M_1):Mitosis:

Observations made in the root tip cells of M_1 seeds revealed chromosome stickiness, clumping and breakage at $M-1$ (Plate-28). Chromosomal abnormalities detectable during mitotic divisions are summarised in Table-161. Details of the observations are as follows:

4 hour treatment:

No cytological abnormality was recorded at 0.2% concentration. At the higher concentration (0.4%), chromosome stickiness, clumping and breakage was observed in 4.0, 6.0 and 4.0 per cent of cells respectively. In the treatment with 0.6 % concentration of EMS solution, increase in the percentage of cells showing chromosome breakage was recorded. When 0.8% solution was used, for 4 hours, 28.0 per cent cells showed increase in chromosome breakage (Plate-28; Figs. 2,3) stickiness and clumping at metaphase and at anaphase bridge (Plate-28; Fig. 8) and bridge + fragment was noticed in 12.0 and 8.0 per cent cells respectively.

8 hours treatment:

In the treatment with 0.2% EMS solution, meiosis followed the normal course. At 0.4% concentration, chromosome breakage (Plate-28; Fig. 4) was noticed in 3.33% cells. At anaphase, bridge with fragment (Plate-28; Fig. 7) was recorded in 3.3% cells. There was corresponding increase in the percentage of cells showing chromosomal changes with the increase in concentration (Table-161) (Plate-28; Fig. 5).

Meiosis: (M₁ plants):

Meiotic studies in M₁ plants revealed multivalents, bivalents and univalents at metaphase-I (Plate-28, 29). Chromosomal configurations at different concentrations and durations of treatments are summarised in Table-162. The details are as follows.

Four hours treatment:0.2 per cent:

At metaphase-I, 11 bivalents formed regularly and no meiotic abnormality was recorded. However, a range of 0-2 univalents with 0.30 per cell was noticed. The pollen fertility percentage was 95.43.

0.4 per cent:

At metaphase-I, frequency of quadrivalents per cell was 0.03 and trivalents 0.01. Ring and rod bivalents ranged from 5-11 and 0-6 with 2.81 and 7.75 per cell respectively. Frequency of univalent per cell was 0.93. At anaphase-I, laggards (Plate-29; Fig. 17) were observed in 4.0% cells and in 2.0% cells delayed separation of one bivalent was noticed. At anaphase-II, 2.0% of cells showed laggards. Sporadic stage comprised tetrads and micronuclei. Pollen fertility was 92.61 per cent (Table-164).

0.6 per cent:

At metaphase-I, quadrivalents ranged from 0-2 with 0.72 per cell and trivalents (Plate-28; Fig. 9) ranged from 0-3 with 0.14 per cell. Ring and rod bivalents ranged from 0-11 and 0-11 with 1.52 and ~~8.52~~ and 8.54 per cell respectively. A range of 0-4 univalents, at

metaphase-I, was recorded, the frequency being 1.09 per cell. At sporad stage other than regular tetrads (Plate-29; Fig. 24) polyads and micronuclei were also noticed. Pollen fertility was 89.42 per cent.

8 hours treatment.

0.2 per cent:

After the treatment with the lowest concentration of EMS solution, normal meiosis was observed. Pollen fertility was 97.1 per cent.

0.4 per cent :

In the treatment with increased concentration multivalents were formed. The frequency of quadrivalents was 0.01 per cell and trivalents 0.02. Ring and rod bivalents ranged from 5-11 and 0-5 with 2.74 and 7.90 per cell respectively. At metaphase-I, univalents (Plate-28; Fig. 14) ranged from 0-4 with 0.55 per cell. At anaphase-I, delayed separation of bivalent was observed in 2.0 per cent cells. At anaphase-II, laggards were recorded in 4.0 per cent cells. At sporad stage tetrads are formed. Occasionally one to two micronuclei were also noticed. Pollen fertility was 90.0 per cent.

0.6 per cent :

At metaphase-I, frequency of quadrivalents (Plate-28; Fig. 13) was 0.01 per cell and trivalents (Plate-28; Fig. 12) 0.09. Ring and rod bivalents ranged from 0-11 and 0-11 with 1.52 and 8.81 per cell respectively. Univalents ranged from 0-4 with 0.5 per cell. At anaphase-I unequal distribution of chromosomes was observed in 2.0% cells (Plate-28; Fig. 15). At anaphase-II also unequal

Table - 159

Germination of EMS treated seeds of Alysicarpus volubilis
 No. of seeds treated in each case was 50.

| Concen- tration (%) | Duration of treat- ment (hours) | Germination in petridish (%) | Emergence of plumule in field (%) | Survival to natu- rity (%) |
|---------------------------|--|------------------------------------|--|-------------------------------------|
| Control | - | 96.0 | 96.84 | 97.82 |
| 0.2 | 4 | 94.0 | 91.39 | 94.11 |
| " | 8 | 90.0 | 88.8 | 92.50 |
| 0.4 | 4 | 80.0 | 81.25 | 82.36 |
| " | 8 | 76.0 | 80.26 | 90.16 |
| 0.6 | 4 | 50.0 | 54.9 | 71.42 |
| " | 8 | 48.0 | 51.0 | 68.0 |
| 0.8 | 4 | 20.0 | 5.0 | NIL |
| " | 8 | 16.0 | 6.0 | NIL |
| 1.0 | 4 | NIL | - | - |
| " | 8 | NIL | - | - |

Morphological observations in control, M₁ and M₂ plants of *Abrusia volubilis*

| Characters | Control | Generation | 4 hours treatment | | | | 8 hours treatment | | | |
|-----------------------------|---------|----------------|-------------------|---------|---------|---------|-------------------|---------|---------|---------|
| | | | 0.2 % | 0.4 % | 0.6 % | 0.2 % | 0.4 % | 0.6 % | 0.2 % | 0.6 % |
| Spread of plant (cm) | 76 | M ₁ | 75.1 | 70.0 | 46.2 | 69.0 | 70.0 | 48.5 | 70.5 | 48.5 |
| | 75 | M ₂ | 76.2 | 72.5 | 70.0 | 71.3 | 72.5 | 70.5 | 70.5 | 70.5 |
| No. of primary branches | 7.6 | M ₁ | 6.5 | 6.4 | 4.2 | 5.8 | 4.7 | 3.8 | 4.7 | 3.8 |
| | 6.5 | M ₂ | 6.6 | 6.8 | 6.0 | 6.0 | 6.5 | 5.7 | 6.5 | 5.7 |
| No. of secondary branches | 11.5 | M ₁ | 10.5 | 9.8 | 8.7 | 9.2 | 9.0 | 8.0 | 9.0 | 8.0 |
| | 10.2 | M ₂ | 11.2 | 10.5 | 10.8 | 11.5 | 9.7 | 8.1 | 9.7 | 8.1 |
| Central leaflet (L x B) cm. | 4.2x4.0 | M ₁ | 4.3x4.1 | 4.2x4.0 | 4.0x3.8 | 4.2x4.1 | 4.5x3.9 | 4.0x3.8 | 4.5x3.9 | 4.0x3.8 |
| | 4.5x4.1 | M ₂ | 4.5x4.2 | 4.3x4.1 | 4.1x3.9 | 4.3x4.1 | 4.6x4.0 | 4.2x4.0 | 4.6x4.0 | 4.2x4.0 |
| Days to flowering | 203 | M ₁ | 201 | 203 | 205 | 202 | 203 | 204 | 203 | 204 |
| | 205 | M ₂ | 204 | 205 | 206 | 205 | 205 | 205 | 205 | 205 |
| Days to maturity | 277 | M ₁ | 275 | 278 | 279 | 277 | 278 | 279 | 278 | 279 |
| | 278 | M ₂ | 278 | 279 | 280 | 278 | 278 | 278 | 278 | 278 |
| Pods per plant | 30.5 | M ₁ | 32.1 | 23.1 | 11.2 | 30.4 | 18.2 | 12.1 | 18.2 | 12.1 |
| | 32.5 | M ₂ | 34.5 | 30.5 | 25.6 | 32.5 | 26.5 | 25.5 | 26.5 | 25.5 |
| Seeds per pod | 2.4 | M ₁ | 2.5 | 2.1 | 1.1 | 2.6 | 2.0 | 1.0 | 2.0 | 1.0 |
| | 2.5 | M ₂ | 2.4 | 2.2 | 2.0 | 2.7 | 2.1 | 2.0 | 2.1 | 2.0 |

* In each generation 10 plants studied

Table - 161

Mitotic observations in M_1 seeds of *Alysiola volubilis* (JM 1984).

| | | M E T A P H A S E | | | | A N A P H A S E | | | | | |
|---------------------------|-------------------------|----------------------------|-----------------------|-----------------------------|-----------------|-----------------|----------------------------|---------------------------|-------------|----------------------|--|
| Concent- ration (%) | Durat- ion (hrs.) | No. of cells studied | Uneffect- ed cells | Chromo- some breakage | Sticki- ness | Clump- ing | No. of cells studied | Normal separa- tion | Bridge | Bridge + fragment | |
| Control | - | 20 | 20 (100) | - | - | - | 25 | 25 (100) | - | - | |
| 0.2 | 4 | 30 | 30 (100) | - | - | - | 20 | 20 (100) | - | - | |
| 0.2 | 8 | 25 | 25 (100) | - | - | - | 25 | 25 (100) | - | - | |
| 0.4 | 4 | 50 | 43 (86.0) | 2 (4.0) | 2 (4.0) | 3 (6.0) | 25 | 24 (96.0) | 1 (4.0) | - | |
| 0.4 | 8 | 30 | 25 (83.3) | 1 (3.33) | 2 (6.66) | 2 (6.66) | 30 | 28 (93.33) | 1 (3.33) | 1 (3.33) | |
| 0.6 | 4 | 50 | 26 (52.0) | 10 (20.0) | 6 (12.0) | 8 (16.0) | 25 | 22 (88.0) | 2 (8.0) | 1 (4.0) | |
| 0.6 | 8 | 40 | 17 (42.5) | 10 (25.0) | 6 (15.0) | 7 (17.5) | 30 | 25 (83.3) | 3 (9.99) | 2 (6.66) | |
| 0.8 | 4 | 50 | 16 (32.0) | 17 (28.0) | 7 (14.0) | 10 (20.0) | 25 | 20 (80.0) | 3 (12.0) | 2 (8.0) | |
| 0.8 | 8 | 25 | 8 (32.0) | 7 (28.0) | 4 (16.0) | 5 (20.0) | 25 | 20 (80.0) | 3 (12.0) | 2 (8.0) | |

(Figures in parentheses are per cent)

33
66
50

Table - 162

Meiotic observations in M_1 plants of *Alysiopsis yulubilis* (JM 1984). No. of plants studied in each case were 5.

| Concen- tration (%) | Dura- tion (hrs.) | No. of cells studied | Chromosomal associations at M-I | | | | | Anaphase - I | | | Anaphase - II | | | Pollen ferti- lity (%) |
|---------------------------|-------------------------|----------------------------|---------------------------------|---------------|----------------|----------------|----------------|----------------------------|-----------------------------|--------------------|--------------------------|----------------------------|--------------------|---------------------------------|
| | | | IV | III | Ring II | Red II | I | No. of cells studied | Dele- ted sep. (%) | Lag- gs. (%) | unequ- al sep. (%) | No. of cells studied | Lag- gs. (%) | |
| Control | - | 50 | - | - | 0-11 (10.6) | 0-1 (0.4) | - | 80 | - | - | - | 95 | - | 99.80 |
| 0.2 | 4 | 40 | - | - | 7-11 (8.35) | 0-4 (2.50) | 0-2 (0.30) | 50 | - | - | - | 50 | - | 96.43 |
| " | 8 | 40 | - | - | 7-11 (8.46) | 0-4 (2.41) | 0-2 (0.25) | 50 | - | - | - | 50 | - | 97.11 |
| 0.4 | 4 | 61 | 0-1 (0.03) | 0-1 (0.01) | 5-11 (2.81) | 0-6 (7.75) | 0-4 (0.93) | 50 | 2.0 | 4.0 | - | 50 | 2.0 | 92.61 |
| " | 8 | 59 | 0-1 (0.01) | 0-1 (0.01) | 5-11 (2.74) | 0-6 (7.90) | 0-4 (0.55) | 50 | 2.0 | - | 2.0 | 50 | 4.0 | 90.0 |
| 0.6 | 4 | 55 | 0-2 (0.72) | 0-3 (0.14) | 0-11 (1.52) | 0-11 (8.54) | 1.09 (1.09) | 61 | 3.27 | 3.27 | 1.63 | 50 | 6.0 | 89.42 |
| " | 8 | 53 | 0-2 (0.01) | 0-3 (0.09) | 0-11 (1.52) | 0-11 (8.81) | 0.50 (0.50) | 50 | 4.0 | 4.0 | 2.0 | 50 | 8.0 | 82.61 |

(figures in parentheses are Mean values)

PLATE - 28 (Effect of EMS on A. volubilis)

Fig. 2-8 Mitosis; 9-15; Meiosis.

Fig. 1. Unifoliate, bifoliate, quadrifoliate with changed phyllotaxy.

Fig. 2. Chromosome breakage at prophase (0.4%)

Fig. 3. Fragmentation at Metaphase (0.4%) (X

Fig. 4. Fragmentation at Metaphase (0.6%) (X 1

Fig. 5. Fragmentation at early Metaphase (0.6%)

Fig. 6. Chromatids, at Anaphase, away from the
(0.6%) (X 1500)

Fig. 7. Multiple bridge + fragments at Anaphase
(X 1500)

Fig. 8. Single chromatid bridge at Anaphase (0.

Fig. 9. 1 III + 8 II's + 3 I's at Metaphase-I (

Fig. 10. 1 VI + 8 II's at Metaphase-I (0.6%) (X

Fig. 11. 11 bivalents showing bipolarity (X 1500)

Fig. 12. 2 III's + 7 II's + 2 I's at Metaphase-I
(X 1500)

Fig. 13. 1 IV + 9 II's at Metaphase (0.6%) (X 15

Fig. 14. 9 II's + 4 I's at Metaphase-I (0.6%) (X

Fig. 15. Unequal distribution of chromosomes (20
(0.6%) (X 1500)

PLATE - 28 (Effect of EMS on A. volubilis)

Fig. 2-8 Mitosis; 9-15; Meiosis.

Fig. 1. Unifoliate, bifoliate, quadrifoliate and leaves with changed phyllotaxy.

Fig. 2. Chromosome breakage at prophase (0.4%) (X 1500)

Fig. 3. Fragmentation at Metaphase (0.4%) (X 1500)

Fig. 4. Fragmentation at Metaphase (0.6%) (X 1500)

Fig. 5. Fragmentation at early Metaphase (0.6%) (X 1500)

Fig. 6. Chromatids, at Anaphase, away from the groups (0.6%) (X 1500)

Fig. 7. Multiple bridge + fragments at Anaphase (0.6%) (X 1500)

Fig. 8. Single chromatid bridge at Anaphase (0.4%) (X 1500)

Fig. 9. 1 III + 8 II's + 3 I's at Metaphase-I (0.4%) (X 1500)

Fig. 10. 1 VI + 8 II's at Metaphase-I (0.6%) (X 1500)

Fig. 11. 11 bivalents showing bipolarity (X 1500)

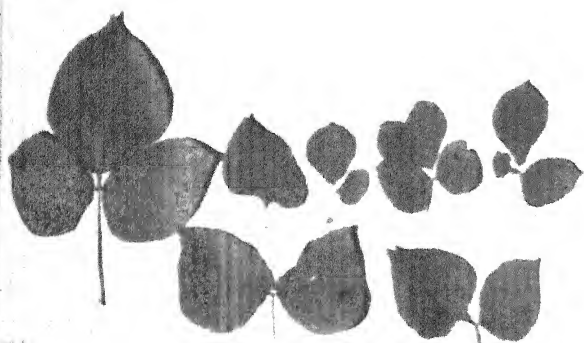
Fig. 12. 2 III's + 7 II's + 2 I's at Metaphase-I (0.6%) (X 1500)

Fig. 13. 1 IV + 9 II's at Metaphase (0.6%) (X 1500)

Fig. 14. 9 II's + 4 I's at Metaphase-I (0.6%) (X 1500)

Fig. 15. Unequal distribution of chromosomes (20-2) (0.6%) (X 1500)

PLATE - 28



1

2

3

4

5

8

10

11

7

6

13

14

9

12

15

PLATE - 29 (Effect of EMS on A. volubilis) : Meiosis

- Fig. 16. Unequal distribution of chromosomes (20-4)
(0.6%) (X 1000)
- Fig. 17. Synthesis at Anaphase-I (0.4%) (X 1000)
- Fig. 18. Unequal distribution at Anaphase-II (0.6%)
(X 1000)
- Fig. 19. Distribution of chromatids in 3 groups
and laggards at Anaphase - II (X 1000)
- Fig. 20. PMC's showing 3 and 4 groups at telophase-II
(X 600)
- Fig. 21. PMC showing disturbed orientation of
chromatids at Anaphase-II (0.6%) (X 1500)
- Fig. 22. Unequal daughter nuclei at telophase-II
(0.6%) (X 1000)
- Fig. 23. Hexad with normal tetrad (0.6%) (X 1000)
- Fig. 24. Dyad with normal tetrad (0.6%) (X 600)
- Fig. 25. Dyad with micronuclei. (0.6%) (X 2800)
- Fig. 26. Sticky groups of pollen grains (X 1500)

PLATE - 29

16

17

18

19

20

21

22

25

23

24

26

distribution of chromatids (Plate-29; Figs, 18, 19) and formation of unequal daughter nuclei was recorded (Plate-29; Fig. 22). At anaphase-I and II, laggards were observed in 4.0 per cent and 8.0 per cent cells respectively. Grouping of chromatids in more than four groups (Plate-29; Fig. 21) was also recorded which resulted in polyad (Plate-29; Fig. 23) formation at sporad stage. Pollen fertility was 82.61 per cent (Table-162).

Effects of EMS on seed germination and plant survival in *Atylosia caianifolia*.

Observations on seed germination in petridished emergence of plumules in field and survival to maturity in 4 and 8 hours treatment at different concentration of EMS solution are as follows:

4 hours treatment:

In contrast to control the lowest concentration (0.2%) of EMS when used for 4 hours showed decline in the percentage seed germination, plumule emergence and plant survival till maturity (Table-163). At 0.4% concentration, seed germination, plumule emergence and plant survival were 60.0, 66.6 and 90.0 per cent respectively. In the treatment with 0.6% further decrease in the seed germination, plumule emergence and plant survival was noticed. At 0.8% concentration, seed germination and plumule emergence were 18.0 and 33.3 per cent respectively. Seedlings could not survive after this treatment.

8 hours treatment:

In the treatment with 0.2% concentration, 78.0 per cent seed germinated, 74.0 percent plumules emerged

and 93.3 per cent plants survived till maturity. At 0.4 and 0.6 per cent, gradual reduction in their percentages was noticed (Table-163). At 0.8% concentration, 14.2 per cent seed germination and 28.5 per cent plumule emergence was recorded but seedlings could not survive after this treatment. The highest concentration of 1.0% EMS solution revealed only 10.0 per cent seed germination, plumules could not emerge after such a treatment.

Gradual reduction in the percentage of seed germination, plumule emergence and plant survival was noticed with increase in the concentration and duration of treatment (Table-163).

Morphological observations in EMS treated plants of *Atylosia caianifolia*.

Morphological observations in control, M_1 and M_2 plants of *A. caianifolia* are summarised in Table-164. Details are as follows:

4 hours treatment:

0.2% :

M_1 plants had 118.6 cm height, 3.7 primary and 6.5 secondary branches. Days to 50% flowering and maturity were nearer to those of control plants (Table-164). Average length of central leaflet was 4.7 cm and breadth 2.1 cm. Pods per plant was 38.0 and seeds per pod 2.7.

M_2 plants had average 119.1 cm height, 3.8 primary and 6.8 secondary branches. Average length of central leaflet was 4.8 and breadth 2.2 cm. Days to 50% flowering and maturity were nearer to those of control plants. On an

average number of pods per plant was 40.0 and seeds per pod 2.8.

0.4 %:

M_1 plants showed 115.0 cm average height 3.8 primary and 6.3 secondary branches. Average length of Central leaflet was 4.5 cm and breadth 2.0 cm. Days to 50% flowering and maturity were 127 and 197 respectively. Pods per plant was 26.0 and seeds per pod 2.1.

M_2 plants had average 118.2 cm height, 3.9 primary and 6.4 secondary branches. Average length of central leaflet was 4.6 cm and breadth 2.1 cm. Days to 50% flowering and maturity were nearer to those of control plants. Pods per plant was 31.3 and seeds per pod 2.2.

0.5 % :

M_1 plants showed 110.5 cm average height. Number of primary and secondary branches were 3.1 and 6.0 respectively. Average length of central leaflet was 4.3 and breadth 2.0 cm. Days to 50% flowering and maturity were 130 and 199 as against 124 x and 195 in control plants. Pods per plant was 14.5 and seeds per pod 1.1.

M_2 plants had average 115.4 cm height, 3.5 primary and 6.5 secondary branches. Average length of central leaflet was 4.4 and breadth 2.1 cm. Days to 50% flowering and maturity were nearer to those of control plants. On average, number of pods per plant was 26.2 and seeds per pod 1.5.

8 hours treatment:

0.2 %:

M_1 plants showed 120.2 cm average height, 3.5

primary and 6.4 secondary branches. Plants showed 4.2 cm average length of central leaflet and 2.0 cm breadth. Days to 50% flowering and maturity 125 and 195. On an average number of pods per plant was 36.5 and seeds per pod 2.6.

M_2 plants had average 118.3 cm average height, 3.6 primary and 6.5 secondary branches. Days to 50% flowering and maturity were nearer to those of control plants. Pods per plant was 38.6 and seeds per pod 2.7.

0.4 %:

M_1 plants showed 116.2 cm average height, 3.4 primary and 6.2 secondary branches. Days to flowering and maturity were 125 and 198. Length of central leaflet was 4.1 cm and breadth 2.0 cm. On an average number of pods per plant was 2.7 and seeds per pod 2.0.

In M_2 plants, 120.3 cm average height, 3.2 primary and 6.3 secondary branches were recorded. Days to 50% flowering and maturity were nearer to those of control plants. On an average pods per plant was 29.8 and seeds per pod 2.3.

0.6 %:

M_1 plant showed 112.3 cm average height, 3.0 primary and 6.1 secondary branches. Average length of central leaflet was 4.0 cm and breadth 2.0 cm. Days to flowering and maturity were 129 and 201 respectively. On an average pods per plant was 16.6 and seeds per pod 1.0.

In M_2 plants 118.2 cm average plant height was observed. Number of primary and secondary branches were 3.1 and 6.2 respectively. Days to 50% flowering and maturity

were nearer to those of control plants. Pods per plant was 20.2 and seeds per pod 1.1.

Cytology M₁:

Mitosis:

Chromosomal abnormalities detectable during mitotic divisions (Plate-30) are summarised in Table-165. Details are as follows:

4 hours treatment:

No cytological abnormality was seen at 0.2% concentration. At the higher concentration (0.4%), chromosome stickiness, clumping and breakage was observed in 4.0, 6.0 per cent cells respectively. At 0.6% strength of the chemical increase in the percentage of cells showing chromosome breakage was recorded (Table-165). When 0.8% EMS solution was applied for 4 hours, stickiness, clumping and chromosome breakage (Plate-30; Fig. 2) were recorded in 10.0%, 20.0 and 22.0 per cent cells recorded. At this concentration anaphasic cells revealed bridge (Plate-30; Fig. 5) without fragments, bridge with fragments and laggards in 12.0, 8.0 and 4.0 per cent cells respectively. At anaphase single, double and multiple bridges were noticed. At metaphase two paired fragments left out of the equatorial plate (Plate-30; Fig. 3) was observed in 6.0% cells. At telophase, formation of formation of 3 groups instead of two (Plate-30; Fig. 6) groups was recorded in 8.0% cells.

8 hours treatment:

At the lowest concentration (0.2% of EMS solution, stickiness and clumping were observed in 4.0 per cent cells

(Table-165). However, at anaphase, no cytological abnormality could be recorded. At the higher concentration (0.4%) increase in chromosome stickiness, clumping (Plate-30; Fig. 7) and breakage was recorded (Table-165). At 0.6% concentration such abnormalities were observed in 8.0, 10.0 and 14.0 per cent cells respectively. At anaphase, bridge with fragment was observed in 10.0 per cent cells. The highest concentration (0.8%) revealed chromosome breakage (Plate-30; Fig. 4) in 20.0 per cent cells. At anaphase, bridge with fragment and without fragment were recorded in 12.0 and 16.0 per cent cells respectively. At this concentration differential condensation of daughter nuclei (Plate-30; Fig. 8) were ^{seen} in 2.5 per cent cells while in 3.5 per cent cells tripolar nuclei were found.

Meiosis:

Chromosome configurations at different concentrations (Plate-30, 31) and durations of treatment (Table-166) are as follows:

4 hours treatment:

0.2% :

At metaphase-I rod bivalents ranged from 0-9 with 2.96 per cell while in untreated plants, from 0-1 with 0.4 per cell. Pollen fertility was 88.72 per cent.

0.4%:

At metaphase-I, trivalents (Plate-30; Fig. 10) ranged from 0-1 with 0.04 per cell. Ring and rod bivalents ranged from 2-11 and 0-9 with 4.62 and 5.81 per cell respectively. Univalents at metaphase-I, ranged from 0-4 with 0.78 per cell. At early anaphase-I delayed separation

Of one bivalent was observed in 2.0% cells, where 4.0% cells showed early separation of two univalents. Laggards were observed in 6.0% cells at anaphase-II. At sporad stage, dyad, triad, tetrad and micronuclei were noticed. Pollen fertility was 82.61 per cent.

0.6 %:

At metaphase-I (Plate-31; Fig. 14) quadrivalents frequency was 0.06 per cell and of trivalents 0.04 per cell. Ring and rod bivalents ranged from 2-11 and 0-9 with 3.30 and 6.38 per cell respectively. At metaphase-I two bivalents left out of the equatorial plate (Plate-30; Fig. 12) was observed in 8.0 per cent cells. And early separation of two univalents was noticed in 7.5% cells. At anaphase-I and II laggards (Plate-31; Fig. 15) were observed in 5.0 and 8.0 per cent cells respectively. At anaphase-II, bridge (Plate-31; Fig. 19) was recorded in 2.5 per cent cells. In some cells formation of more than 4 groups, at anaphase-II, was also noticed. At sporad stage other than tetrads, dyads, triad, polyad and micronuclei were observed. Pollen fertility was 57.22 per cent.

8 hours treatment:

0.2 %:

At metaphase-I, ring and rod bivalents ranged from 2-11 and 0-9 with 8.20 and 2.50 per cell respectively. At metaphase-I two univalents, left out of the equatorial plate (Plate-30; Fig. 9) was notice in 4.0 per cent cells. At sporad stage regular tetrad formation was observed. Pollen fertility was 86.81%.

Table - 165

Mitotic observations in M_1 seeds of *Atylosia calanifolia*

| Concentration (%) | Duration of treatment (hrs.) | METAPHASE | | | | ANAPHASE | | | |
|-------------------|------------------------------|----------------------|------------------|---------------------|-------------|--------------|----------------------|-------------------|-------------------|
| | | No. of cells studied | Unaffected cells | Chromosome breakage | Stickiness | Clumping | No. of cells studied | Normal separation | Bridge + fragment |
| Control | - | 20 | 20 (100) | - | - | - | 25 | 25 (100) | - |
| 0.2 | 4 | 30 | 28 (93.33) | - | 1 (3.33) | 1 (3.3) | 20 | 20 (100) | - |
| " | 8 | 25 | 23 (92.0) | - | 1 (4.0) | 1 (4.0) | 25 | 25 (100) | - |
| 0.4 | 4 | 50 | 42 (84.0) | 3 (6.0) | 2 (4.0) | 3 (6.0) | 30 | 27 (90.0) | 1 (3.3) |
| " | 8 | 35 | 29 (82.85) | 2 (5.71) | 2 (5.71) | 2 (5.71) | 25 | 22 (88.0) | 1 (4.0) |
| 0.6 | 4 | 50 | 37 (74.0) | 6 (12.0) | 3 (6.0) | 4 (8.0) | 25 | 20 (80.0) | 2 (8.0) |
| " | 8 | 50 | 32 (64.0) | 7 (14.0) | 6 (8.0) | 5 (10.0) | 30 | 25 (83.3) | 3 (10.0) |
| 0.8 | 4 | 50 | 24 (48.0) | 11 (22.0) | 5 (10.0) | 10 (20.0) | 25 | 19 (76.0) | 2 (8.0) |
| " | 8 | 20 | 10 (50.0) | 4 (20.0) | 2 (10.2) | 4 (20.0) | 25 | 18 (72.0) | 3 (12.0) |

(Figures in parentheses are per cent)

Table - 166

Meiotic observations in M_1 plants of *Alylosia cajaniifolia* (No. of plants studied in each case were 5)

| Concen- tration (%) | Dura- tion (hrs) | No. of cells studied | Chromosomal associations at M-I | | | | | Anaphase - I | | | | Anaphase - II | | | | Pollen ferti- lity (%) |
|---------------------------|------------------------|----------------------------|---------------------------------|---------------|-----------------|---------------|---------------|----------------------------|----------------------|-------------------|-----------------------------|----------------------------|----------------------------|-------------------|-------------------|---------------------------------|
| | | | IV | III | Ring II | Rad II | I | No. of cells studied | Early sep. (%) | Lag- gs (%) | De- layed sep. (%) | No. of cells studied | No. of cells studied | Lag- gs (%) | Lag- gs (%) | |
| Control | - | 50 | - | - | 10-11 (10.6) | 0-1 (0.4) | - | 50 | - | - | - | 60 | - | - | - | 99.2 |
| 0.2 | 4 | 50 | - | - | 2-11 (7.84) | 0-9 (2.96) | 0-2 (0.4) | 50 | 2.0 | - | - | 33 | - | - | 3.0 | 88.72 |
| " | 8 | 52 | - | - | 2-11 (8.20) | 0-9 (2.80) | 0-2 (0.38) | 50 | 2.0 | 4.0 | - | 50 | - | - | 4.0 | 86.81 |
| 0.4 | 4 | 43 | - | 0-1 (0.04) | 2-11 (4.62) | 0-9 (5.81) | 0-4 (0.74) | 50 | 4.0 | - | 2.0 | 50 | - | - | 6.0 | 82.61 |
| " | 8 | 42 | - | 0-1 (0.02) | 2-11 (4.76) | 0-9 (5.90) | 0-4 (0.66) | 50 | 4.0 | - | 2.0 | 50 | - | - | 6.0 | 80.55 |
| 0.6 | 4 | 50 | 0-1 (0.06) | 0-2 (0.14) | 2-11 (3.30) | 0-9 (6.38) | 0-6 (1.86) | 40 | 7.5 | 5.0 | - | 40 | 2.5 | 8.0 | - | 57.22 |
| " | 8 | 43 | 0-1 (0.04) | 0-2 (0.05) | 3-11 (4.24) | 0-8 (6.55) | 0-6 (1.55) | 50 | 10.0 | 4.0 | 2.0 | 50 | - | 8.0 | - | 51.66 |

(Mean values in parentheses)

Table - 163

Germination of EMS treated seeds of Atylosia cajanifolia
 No. of seeds treated in each case was 50.

| Concen- tration (%) | Dura- tion of treat- ment (hours) | Germi- nation in petri- dish (%) | Emergence of plumule in field (%) | Survival to matu- rity (%) |
|---------------------------|---|---|--|----------------------------------|
| Control | - | 100 | 98.0 | 97.91 |
| 0.2 | 4 | 80.0 | 72.0 | 93.75 |
| " | 8 | 78.0 | 74.0 | 93.3 |
| 0.4 | 4 | 60.0 | 66.6 | 90.0 |
| " | 8 | 58.0 | 62.0 | 83.3 |
| 0.6 | 4 | 40.0 | 60.0 | 82.3 |
| " | 8 | 36.0 | 61.1 | 72.0 |
| 0.8 | 4 | 18.0 | 33.3 | NIL |
| " | 8 | 14.2 | 28.5 | NIL |
| 1.0 | 4 | 12.0 | NIL | - |
| " | 8 | 10.0 | NIL | - |

Morphological observations in control, M₁ and M₂ plants of *Alysicarpus eschscholzii*

| Characters | Control | Gene- ration * | 4 hours treatment | | | 8 hours treatment | | |
|----------------------------|---------|----------------------|-------------------|---------|---------|-------------------|---------|---------|
| | | | 0.2% | 0.4% | 0.6% | 0.2% | 0.4% | 0.6% |
| Height of plant (cm) | 120 | M ₁ | 118.5 | 115.0 | 110.5 | 120.2 | 116.2 | 112.4 |
| | 117 | M ₂ | 119.1 | 118.2 | 115.4 | 118.3 | 112.3 | 118.2 |
| No. of primary branches | 4.5 | M ₁ | 3.7 | 3.8 | 3.1 | 3.5 | 3.4 | 3.0 |
| | 3.2 | M ₂ | 3.8 | 3.9 | 3.5 | 3.6 | 3.2 | 3.1 |
| No. of secondary branches | 7.3 | M ₁ | 6.5 | 6.3 | 6.0 | 6.4 | 6.2 | 6.1 |
| | 6.5 | M ₂ | 6.8 | 6.4 | 6.5 | 6.5 | 6.3 | 6.2 |
| Central leaflet (L x B) cm | 4.8x2.1 | M ₁ | 4.7x2.1 | 4.5x2.0 | 4.2x2.0 | 4.2x2.0 | 4.1x2.0 | 4.0x2.0 |
| | 4.7x2.0 | M ₂ | 4.8x2.2 | 4.6x2.1 | 4.4x2.1 | 4.3x2.1 | 4.2x2.1 | 4.2x2.1 |
| Days to flowering | 124 | M ₁ | 124 | 127 | 130 | 125 | 125 | 129 |
| | 122 | M ₂ | 120 | 123 | 124 | 123 | 124 | 127 |
| Days to maturity | 195 | M ₁ | 194 | 196 | 199 | 195 | 198 | 201 |
| | 190 | M ₂ | 191 | 196 | 198 | 190 | 196 | 199 |
| Pods per plant | 40.0 | M ₁ | 38.0 | 26.0 | 14.5 | 36.5 | 27.1 | 16.6 |
| | 38.0 | M ₂ | 40.0 | 31.3 | 26.2 | 38.6 | 29.8 | 20.2 |
| Seeds per pod | 2.8 | M ₁ | 2.7 | 2.1 | 1.1 | 2.6 | 2.0 | 1.0 |
| | 2.6 | M ₂ | 2.8 | 2.2 | 1.5 | 2.7 | 2.3 | 1.1 |

* In each generation number of plants studied were 5

PLATE - 30 (Effect of EMS on A. cajanifolia)

Fig. 2-8: Mitosis, 9-12: Meiosis.

Fig. 1. Bifoliate, trifoliate and quadrifoliate leaves of A. cajanifolia (0.6%)

Fig. 2. Chromosome breakage at prophase (0.4%)

Fig. 3. Fragmentation at Metaphase (0.8%), two paired fragments away from the equational plate (X 1500)

Fig. 4. Fragmentation at Metaphase-I (0.8%) (X 1500)

Fig. 5. Bridge at Anaphase-(0.8%) (X 1500)

Fig. 6. Formation of 3 unequal groups at telophase (X 1500)

Fig. 7. Extremely clumped chromosome fragments (0.8%) (X 1500)

Fig. 8. Condensed and non-condensed interphase nuclei. (0.8%) (X 1000)

Fig. 9. 10 II's + 2 I's at Metaphase-I, 2 I's away from the equational plate (0.4%) (X 1500)

Fig. 10. 1 III + 8 II's + 3 I's at Metaphase-I one univalent away from the group (0.4%) (X 1500)

Fig. 11. Sticky bivalents at Metaphase-I forming 3 groups (0.6%) (X 1500)

Fig. 12. 11 II's at Metaphase-I, two bivalents away from the groups (0.6%) (X 1500) 7

PLATE - 30



1



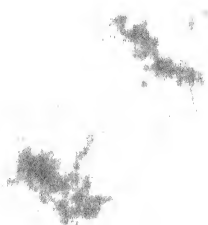
3



4



2



5



6



7



8



9



11



12



10

PLATE - 31 (Effect of EMS on A. cajanifolia : Meiosis)

Fig. 13. 1 IV + 9 II's (0.4%) (x1000)

Fig. 14. 1 IV + 8 II's + 2 I's at Metaphase-I (0.6%)
(x 1500)

Fig. 15. Laggards at Anaphase-I (0.6%) (x 1500)

Fig. 16. Delayed separation of one bivalent at
Metaphase-I (0.6%) (x 1500)

Fig. 17. Laggards at Anaphase-II (0.6%) (x 1500)

Fig. 18. Chromatids in 6 groups at Anaphase-II
(0.6%) (x1000)

Fig. 19. Single chromatid bridge at Anaphase-II
(0.6%) (x 1500)

Fig. 20. Dyad with normal tetrads (x 600)

Fig. 21. Hexad with normal tetrad (0.6%) (x 600)

Fig. 22. Fertile pollen grains showing variation in
size (0.6%) (x 600)

PLATE - 31



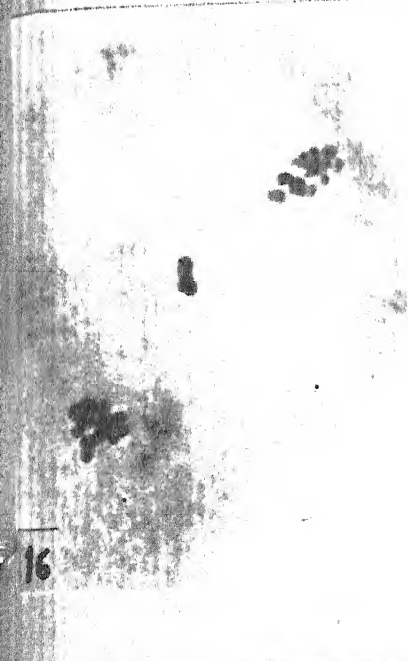
13



14



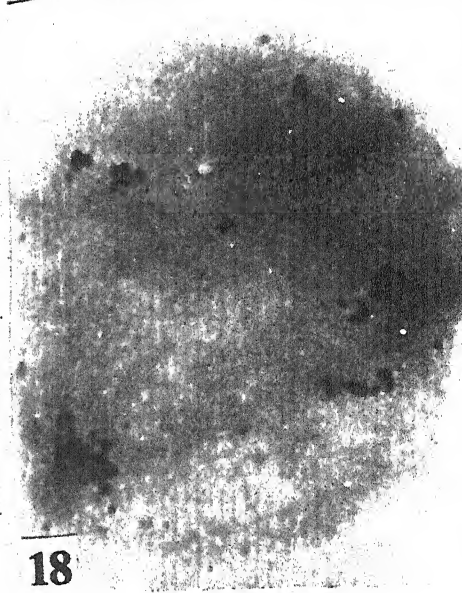
15



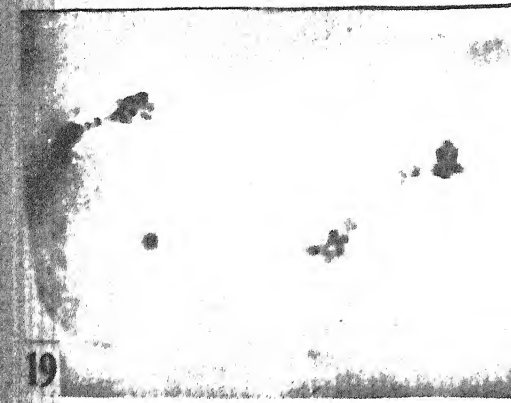
16



17



18



19



20



21



22

0.4 %:

At metaphase-I, trivalents frequency was 0.02 per cell. Ring and rod bivalents ranged from 2-11 and 0-4 with 4.76 and 5.90 per cell respectively. Two univalents left out of the equatorial plate was noticed in 10.0 per cent cells. At anaphase-I, 2.0 per cent cells showed delayed separation of one bivalent (Plate-31; Fig.16). At anaphase-II, laggards were recorded in 6.0 per cent cells. At sporad stage, other than tetrads, triad and micronuclei were also recorded. Pollen fertility was 80.55 per cent.

0.6 %:

At metaphase-I, quadrivalents (Plate-31; Fig. 13) frequency was 0.04 per cell and of trivalent 0.05 per cell. Ring bivalents ranged from 3-11 with 4.24 per cell and rod bivalents ranged from 0-8 with 6.55 per cell. Univalents, at metaphase-I, ranged from 0-6 with 1.55 per cell. In 8.0% cells, two bivalents left out of the equatorial plate of metaphase was noticed. At metaphase-I early separation of two univalents was noticed in 12.0 per cent cells. Laggards at anaphase-I and II was observed in 4.0 8.0 per cent cells respectively. At sporad stage dyad, triad, tetrad, polyad (Plate-31; Figs, 20,21) and micronuclei were noticed. Pollen fertility was 51.66 per cent.

Effects of EMS on seed germination and plant survival in *Alysicia albicans*.

Gradual reduction in the percentage of seed germination and plant survival was noticed with increase in concentration and duration of treatment (Table-167). Observations on seed germination in petridishes, emergence

of plumules in the field and survival to maturity in 4 and 8 hours treatments, at different concentrations are as follows:

Four hours treatment:

After the treatment with the lowest concentration (0.2%) of EMS solution for 4 hours 90.0% seed germination, 77.2% plumule emergence in field and 85.2% plant survival to maturity was recorded (Table-167). At 0.4% concentration, seed germination, plumule emergence and survival to maturity were 80.0%, 75.0% and 84.9% respectively. After the treatments with 0.6 and 0.8 per cent EMS solutions reduction in the percentage of seed germination, plumule emergence and survival to maturity was noticed (Table-167). In the highest concentration of 1.0% EMS treatment, only 20.0% seeds germinated and plumule could not emerge due to toxic effects of the chemical, thus no seedling was raised.

Eight hours treatment:

At 0.2% concentration, 88.0% seeds germinated, 79.54 plumules emerged and 85.7% plants survived (Table-167). 0.4% EMS solution when used for the period of 8 hours percentage seed germination, plumule emergence and plant survival were 76.0, 74.0 and 84.2 respectively. In the treatments with 0.6% and 0.8% EMS solutions gradual reduction in percentage of seed germination, plumule emergence and plant survival was noticed (Table-167). At the highest concentration of EMS solution (1.0%) only 12.0% seed germination was recorded. Plumules could not emerge after such a treatment (Table-167).

Morphological observations in EMS treated plants of *Alysicarpus albus*.

Studies on different morphological characters were

recorded in EMS treated plants and compared with those of control (Table-168). Morphological observations at various concentrations and durations are as follows:

a) Four hour treatment:

(i) 0.2%:

M_1 plants showed 45.1 cm average plant spread as against 65.0 cm in control. On an average, the number of primary and secondary branches were 8.0 and 9.2 respectively. In M_1 plants, average length and breadth of central leaflet was 4.0 cm and 3.1 cm respectively. Days to 50% flowering and maturity were 139 and 204 in M_1 plants as against 130 and 202 in control. Pods per plant and seeds per pod were 32.5 and 2.1 respectively.

In M_2 plants, 68.5 cm average plant spread was recorded (Table-168). Number of primary and secondary branches were 9.1 and 11.2 respectively. Days to 50% flowering and maturity were nearer to those of control. Average length and breadth of central leaflet was 4.2 cm and 3.1 cm in M_2 plants. Pods per plant and seeds per pod was 36.1 and 2.2 respectively.

(ii) 0.4 %:

M_1 plant showed, on an average, 37.6 cm plant spread and 8.5 primary and 8.3 secondary branches. Length and breadth of central leaflet was 3.9 cm and 3.0 cm respectively. Days to 50% flowering and maturity were 140 and 210 respectively. In M_1 plants 28.2 pods per plant and 20 seeds per pod was recorded.

In M_2 plants on an average 59.2 cm plant spread, 9.2 primary branches and 12.4 secondary branches was recorded.

Average length and breadth of central leaflet was 4.5 cm and 3.5 cm respectively. Days to 50% flowering and maturity were nearest to these of control (Table-168). Pods per plant and average number of seeds per pod were 30.4 and 2.1 respectively.

(iii) 0.6% :

M_1 plants showed 31.3 cm average plant spread and 7.3 and 8.3 primary and secondary branches. Length and breadth of central leaflet were 3.8 cm and 2.9 cm respectively. Days to 50% flowering and maturity were 141 and 211 as against 128 and 202 in control respectively. Pods per plant was 18.4 and seeds per pod 1.9.

M_2 plants showed 37.7 cm average plant spread. The number of primary and secondary branches on an average were 8.1 and 11.0 respectively. Average central leaflet length was 4.5 cm and breadth 3.2 cm. Days to 50% flowering and maturity were 142 and 212 respectively. The number of pods per plant was 35.1 and seeds per pod 2.2.

(iv) 0.8% :

M_1 plants showed 25.3 cm average spread as against 65.0 cm in control. Number of primary and secondary branches were 7.4 and 7.0 respectively. Length and breadth of central leaflet were 3.9 cm and 2.8 cm respectively. Days to flowering and maturity were 143 and 211. Average number of pods per plant and seeds per pod were 9.1 and 1.2 respectively.

Eight hour treatment:

0.2% :

The M_1 plants showed 48.1 cm average plant spread.

Number of primary and secondary branches were 6.8 and 7.8 respectively. On an average, M_1 plants showed 4.1 cm leaf length and 2.2 cm leaf breadth. Days to 50% flowering and maturity were nearer to those of control plants of A. albicans (Table-168). Average number of pods per plant and seeds per pod were 33.0 and 1.9 respectively.

M_2 plants showed 50.3 cm average plant spread and 6.8 and 9.5, primary and secondary branches respectively. Length and breadth of central leaflet were 4.1 cm and 3.2 cm respectively. Days to 50% flowering and maturity ^{were} nearer to those of control plants (Table-168). Number of pods per plant and seeds per pod were 35.7 and 2.0 respectively.

0.4 % i

M_1 plants showed 41.2 cm average plant spread. Number of primary and secondary branches were 7.0 and 7.9 respectively. Average leaf length was 4.1 cm and breadth 3.1 cm. Days to 50% flowering and maturity were nearer to those of control plants (Table-168). Average number of pods per plant and seeds per pod were 30.1 and 2.0 respectively.

0.6 % i

M_1 plants on an average showed 40.4 cm plant spread and 6.0 primary and 8.0 secondary branches (Table-168). Average length and breadth of central leaflet were 3.9 cm and 3.2 cm respectively. Days to 50% flowering and maturity were 139 and 209 respectively. Number of pods per plant was 29.8 and seeds per pod 1.7.

Average plant spread in M_2 's was 47.4 cm and primary and secondary branches were 6.8 and 10.3 respectively. Average central leaflet length was 3.9 cm and breadth 2.32 cm. Days to 50% flowering and maturity were nearer to those of control plants (Table-168). Number of pods per plant was 30.0 and seeds per pod 2.0.

0.8 % :

M_1 plants raised after 0.8% EMS treatment showed average 26.0 cm plant spread. Average number of primary and secondary branches were 5.3 and 6.7 respectively. Average leaf length was 3.8 cm and breadth was 3.0 cm. Days to 50% flowering and maturity were 141 and 210 respectively. Number of pods per plant and seeds per pod were 8.5 and 1.3 respectively (Table-168).

M_2 plants showed increased plant spread (36.0 cm) over M_1 plants (Table-168). Number of primary and secondary branches were 7.3 and 9.1 respectively and leaflength and breadth were 4.2 cm and 3.0 cm. Days to 50% flowering and maturity were nearer to those of control plants. On an average, number of pods per plant and seeds per pod were 22.5 and 1.8 respectively, showing an increase over M_1 plants.

Cytology (M_1):

Mitotic abnormalities at somatic metaphase of M_1 plants showed chromosome stickiness, clumping and breakage (Plate-32). Chromosomal abnormalities during mitotic divisions are summarised in Table-169. Details are as follows.

4 hour treatment:

Mitosis was normal in the treatment with 0.2 and 0.4 per cent EMS solution. At higher concentration (0.6%) somatic cells revealed stickiness, clumping and chromosome break in 3.3, 5.0 and 5.0 per cent cells respectively. At anaphase bridge without fragment and bridge with fragment was noticed in 4.0 and 2.0 per cent cells respectively. The highest concentrations of EMS used for 4 hours revealed stickiness in 10.0 per cent cells, clumping in 12.0 per cent cells and chromosome breakage (Plate-32; Fig. 2) in 18.0 per cent cells. At anaphase bridge without fragment and bridge with fragment was noticed in 10.0 and 4.0 per cent cells respectively.

8 hour treatment:

No cytological abnormality was recorded in the treatment with 0.2%. At 0.4% concentration, stickiness of chromosomes was noticed in 4.0 per cent cells. When 0.6% EMS solution was used for 8 hours, showed stickiness, clumping and chromosome breakage were observed in 6.0, 10.0 and 12.0 per cent cells respectively. At anaphase bridge was observed in 8.0 and 4.0 per cent cells bridge with fragment (Plate-32; Fig. 6) was noticed. The highest concentration of (0.8%) EMS solution revealed 20.0 per cent chromosome breakage (Plate-32; Fig. 3) and subsequent increase in stickiness and clumping of chromosomes. At anaphase-I, laggards (Plate-32; Fig. 5) and bridge were observed in 4.0 and 8.0 per cent cells respectively (Table-169).

Meiosis (M_1 plants):

Meiotic studies in M_1 plants revealed trivalents, bivalents and univalents at metaphase-I (Plate-32; 35).

It can be seen from the Table-170 that gradual increase in the frequency of rod bivalents and decrease in ring bivalents, with increase in concentrations was noticed. Observations on chromosomal associations at each concentration and duration are as follows:

4 hour treatment:

0.2 %:

The treatment with 0.2% for 4 hours revealed bivalents and univalents at metaphase-I. Ring bivalents ranged from 5-11 with 7.24 per cell and rod bivalents ranged from 0-6 with 3.64 per cell. At anaphase-I and II, regular separation of equal chromosomes to the poles was observed, at sporad stage tetrads were formed in all the cells studied resulting in high pollen fertility (96.4%).

0.4 %:

At metaphase-I, ring bivalents ranged from 3-11 with 4.33 per cell and rod bivalents ranged from 0-8 with 6.33 per cell. Univalents (Plate-32; Fig. 10) ranged from 0-4 with 0.49 per cell (Table-170). At anaphase-I delayed separation of one/two bivalents and laggards was observed in 2.2 and 2.2 per cent cells respectively. At anaphase-II equal separation of chromatids to the poles was observed in all the cells studied, resulting in regular tetrad formation at sporad stage and high pollen fertility (93.8%).

0.6 %:

At metaphase-I, ring and rod bivalents ranged from 0-11 and 0-11 with 1.51 and 8.95 per cell respectively.

At the same stage trivalents ranged from 0-1 with 0.04 per cell and univalents ranged from 0-6 with 0.93 per cell (Table-170). At anaphase-I, delayed separation of bivalent and laggards (Plate-33; Figs. 14, 15) were observed in 4.0 and 2.0 per cent cells respectively. At anaphase-II, laggards were noticed in 2.0% cells. Regular tetrad formation was observed resulting in high pollen fertility (91.7%).

0.8 %:

At metaphase-I, ring bivalents ranged from 0-11 with 1.15 per cell and rod bivalents (Plate-32; Fig. 12) ranged from 0-11 with 9.04 per cell. Trivalents and univalents ranged from 0-1 and 0-6 with 1.06 and 1.40 per cell respectively (Table-170). At anaphase-I delayed separation of bivalent and laggards were observed in 4.0 and 6.0 per cent cells respectively. At anaphase-II, laggards and non-orientation of chromatids (Plate-33; Fig. 17) was observed in 2.0 and 4.0 per cent cells respectively. At sporad stage other than tetrads dyad (Plate-33; fig. 18) and triad with micronuclei were recorded. Pollen fertility percentage was 85.6.

8 hour treatment:

0.2 %:

At metaphase-I, ring bivalents ranged from 6-11 with 7.33 per cell and rod bivalents ranged from 0-5 with 3.55 per cell (Table-170). Univalent ranged from 0-2 with 0.23 per cell. At anaphase-I and II equal separation of chromosomes to the poles was recorded in all the cells, resulting in regular tetrad formation and high pollen fertility (95.0%).

0.4 X :

At metaphase-I, ring bivalents ranged from 4-11 with 4.25 per cell and rod bivalents ranged from 0-7 with 6.50 per cell. Univalents ranged from 0-4 with 0.49 per cell (Table-170). At anaphase-I delayed separation of one bivalent and laggards were observed in 2.0 and 2.0 per cent cells respectively. At anaphase-I, regular separation of equal chromosomes was noticed. At sporad stage tetrads were observed in all the cells studied, resulting in high pollen fertility (92.5%).

0.6 X :

At metaphase-I, trivalents ranged from 0-1 with 0.30 per cell. Ring and rod bivalents ranged from 0-11, and 0-11 with 2.20 and 7.99 per cell respectively. At the same stage univalents ranged from 0-6 with 0.87 per cell (Table-170). At anaphase-I, delayed separation of one bivalent and laggards were observed in 4.0 and 4.0 per cent cells respectively. At anaphase-II, laggards were observed in 2.0 percent cells. At sporad stage, tetrad formation was observed except in few cells where traid (Plate-33; Fig. 19) formation was noticed. Pollen fertility was 90.2 per cent.

0.8 X :

At metaphase-I, trivalent ranged from 0-1 with 0.04 per cell. Ring and rod bivalents (Plate-32; Fig. 11) ranged from 0-11 and 0-11 with 1.12 and 9.00 per cell respectively. At the same stage, univalents ranged from 0-9 with 1.65 per cell (Table-170). At anaphase-I, delayed separation of bivalent and formation of laggards were noticed in 4.0 and 8.0 per cent cells respectively. At anaphase-II, laggards were observed in 4.0 per cent cells. At sporad stage dyad,

Table - 167

Germination of EMS treated seeds of Atylosia albicans.
No. of seeds treated in each case was 50.

| Concen- tration (%) | Dura- tion of treat- ment (Hours) | Germination in petri- dish (%) | Emergence of plumule in field (%) | Survival to maturity (%) |
|-----------------------------|---|--|--|----------------------------------|
| Control | - | 98.0 | 94.0 | 92.0 |
| 0.2 | 4 | 90.0 | 77.2 | 85.2 |
| 0.2 | 8 | 88.0 | 79.54 | 85.7 |
| 0.4 | 4 | 80.0 | 75.0 | 84.8 |
| 0.4 | 8 | 76.0 | 74.0 | 84.2 |
| 0.6 | 4 | 70.0 | 74.2 | 76.6 |
| 0.6 | 8 | 66.0 | 74.6 | 74.0 |
| 0.8 | 4 | 60.0 | 50.0 | 50.0 |
| 0.8 | 8 | 50.0 | 48.0 | 50.0 |
| 1.0 | 4 | 20.0 | NIL | - |
| 1.0 | 8 | 12.0 | NIL | - |

| Characters | Control | Gene- ration † | 4 hours treatments | | | | 8 hours treatments | | | |
|------------------------------|---------|----------------------|--------------------|---------|---------|---------|--------------------|---------|---------|---------|
| | | | 0.2% | 0.4% | 0.6% | 0.8% | 0.2% | 0.4% | 0.6% | 0.8% |
| Plants spread (cm.) | 65 | M ₁ | 45.1 | 37.6 | 31.1 | 25.3 | 43.1 | 41.2 | 40.4 | 26.0 |
| | 62 | M ₂ | 68.5 | 59.2 | 57.7 | 50.5 | 50.3 | 49.2 | 47.4 | 36.0 |
| No. of primary branches | 8.5 | M ₁ | 8.0 | 8.5 | 7.3 | 7.4 | 6.8 | 7.0 | 6.9 | 5.3 |
| | 9.0 | M ₂ | 9.1 | 9.2 | 8.1 | 8.2 | 7.1 | 7.3 | 6.8 | 7.3 |
| No. of secondary branches | 11.1 | M ₁ | 9.2 | 8.3 | 8.3 | 7.0 | 7.8 | 7.9 | 8.0 | 6.7 |
| | 12.0 | M ₂ | 11.2 | 12.4 | 11.0 | 9.5 | 9.5 | 10.5 | 10.3 | 9.1 |
| Central leaflet (lvs) cm | 4.0x3.0 | M ₁ | 4.0x3.1 | 3.9x3.0 | 3.8x2.9 | 3.9x2.8 | 4.1x3.2 | 4.1x3.1 | 3.9x3.2 | 3.8x3.0 |
| | 4.2x3.3 | M ₂ | 4.2x3.1 | 4.5x3.5 | 4.5x3.2 | 4.1x3.5 | 4.6x3.4 | 4.0x3.2 | 4.0x3.0 | 4.2x3.0 |
| Days to flowering | 138 | M ₁ | 139 | 140 | 141 | 143 | 137 | 138 | 139 | 141 |
| | 140 | M ₂ | 140 | 142 | 142 | 143 | 140 | 141 | 141 | 144 |
| Days to maturity | 202 | M ₁ | 204 | 210 | 211 | 211 | 202 | 206 | 209 | 211 |
| | 208 | M ₂ | 208 | 210 | 212 | 212 | 209 | 208 | 210 | 210 |
| Pods per plant | 34 | M ₁ | 32.5 | 28.2 | 18.4 | 9.1 | 33.0 | 30.1 | 29.8 | 8.5 |
| | 35 | M ₂ | 36.1 | 30.4 | 35.1 | 29.3 | 35.7 | 32.6 | 30.0 | 22.5 |
| Seeds per pod | 2.3 | M ₁ | 2.1 | 2.0 | 1.9 | 1.2 | 1.9 | 2.0 | 1.7 | 1.3 |
| | 2.2 | M ₂ | 2.2 | 2.1 | 2.2 | 1.8 | 2.0 | 2.1 | 2.0 | 1.8 |

* In each generation 10 plants were studied.

Table - 169
Mitotic observations in M_1 seeds of Atriplosia albicans.

| | | M E T A P H A S E | | | | A N A P H A S E | | | | |
|---------------------------|-------------------------|----------------------------|--------------------------------|-----------------------------|-----------------|-----------------|----------------------------|---------------------------|--------------------|--------------------|
| Concent- ration (%) | Durat- ion (hrs.) | No. of cells studied | Unaffected cells Zn = 22 | Chromo- some breakage | sticki- ness | Clump- ing | No. of cells studied | Normal separa- tion | Bridge fragment | Bridge + Laggs. |
| Control | - | 25 | 25 (100) | - | - | - | 25 | 25 (100) | - | - |
| 0.2 | 4 | 30 | 30 (100) | - | - | - | 30 | 30 (100) | - | - |
| 0.2 | 8 | 25 | 25 (100) | - | - | - | 30 | 30 (100) | - | - |
| 0.4 | 4 | 20 | 20 (100) | - | - | - | 30 | 30 (100) | - | - |
| 0.4 | 8 | 25 | 23 (96.0) | - | 1 (4.0) | - | 40 | 39 (97.5) | 1 (2.5) | - |
| 0.6 | 4 | 60 | 52 (86.66) | 3 (5.0) | 2 (3.33) | 3 (5.0) | 50 | 47 (94.0) | 2 (4.0) | 1 (2.0) |
| 0.6 | 8 | 50 | 36 (72.0) | 6 (12.0) | 3 (6.0) | 5 (10.0) | 25 | 22 (88.0) | 2 (8.0) | 1 (4.0) |
| 0.8 | 4 | 50 | 30 (60.0) | 9 (18.0) | 5 (10.0) | 6 (12.0) | 50 | 43 (86.0) | 5 (10.0) | 2 (4.0) |
| 0.8 | 8 | 50 | 27 (54.0) | 10 (20.0) | 6 (12.0) | 7 (14.0) | 25 | 20 (80.0) | 2 (8.0) | 1 (4.0) |

(Figures in parentheses are per cent)

Table - 170

Meiotic observations in M_1 plant of *Atylosia albicans* (No. of plants studied in each case were 5)

| Concen- tration (%) | Dura- tion (hrs.) | M E T A P H A S E - I | | | | | ANAPHASE - I | | ANAPHASE - II | | Pollen ferti- lity (%) | |
|---------------------------|-------------------------|----------------------------|-----------------------------------|----------------|----------------|----------------------------|-----------------------------|--------------------|----------------------------|--------------------|---------------------------------|------|
| | | No. of cells studied | Chromosome Associations at M-I | | | No. of cells studied | Dele- yed sep. (%) | Lag- gs. (%) | No. of cells studied | Lag- gs. (%) | | |
| | | | III | Ring | Rod | | | | | | | |
| | | | | II | II | | | | | | | I |
| Control | - | 60 | - | 9-11 (10.3) | 0-2 (0.7) | - | - | 80 | - | 50 | - | 96.6 |
| 0.2 | 4 | 50 | - | 5-11 (7.24) | 0-6 (3.64) | 0-2 (0.36) | - | 60 | - | 50 | - | 96.4 |
| " | 8 | 51 | - | 6-11 (7.33) | 0-5 (3.55) | 0-2 (0.23) | - | 70 | - | 50 | - | 95.0 |
| 0.4 | 4 | 60 | - | 3-11 (4.33) | 0-8 (6.33) | 0-4 (0.49) | 2.22 | 45 | 2.22 | 50 | - | 93.8 |
| " | 8 | 57 | - | 4-11 (4.25) | 0-7 (6.50) | 0-4 (0.49) | 2.0 | 50 | 2.0 | 50 | - | 92.5 |
| 0.6 | 4 | 43 | 0-1 (0.04) | 0-11 (3.51) | 0-11 (8.25) | 0-6 (0.93) | 4.0 | 50 | 4.0 | 50 | 2.0 | 91.7 |
| " | 8 | 31 | 0-1 (0.03) | 0-11 (2.20) | 0-11 (7.99) | 0-6 (0.87) | 4.0 | 50 | 4.0 | 50 | 2.0 | 90.2 |
| 0.8 | 4 | 45 | 0-1 (1.06) | 0-11 (1.15) | 0-11 (9.04) | 0-6 (1.40) | 4.0 | 50 | 4.0 | 50 | 2.0 | 85.6 |
| " | 8 | 41 | 0-1 (0.04) | 0-11 (1.12) | 0-11 (9.00) | 0-9 (1.65) | 4.0 | 50 | 4.0 | 50 | 4.0 | 80.0 |

(Mean values in parentheses)

PLATE - 32 (Effect of EMS on A. albicans)

Fig. 2-7: Mitosis, 8-12: Meiosis.

Fig. 1. Morphological variations in leaflet number and shape.

Fig. 2. Chromosome breakage at prophase (0.4%) (X 1500)

Fig. 3. Chromosome breakage at Metaphase (0.8%) (X 1500)

Fig. 4. Lagging fragments at Anaphase (0.6%) (X 1500)

Fig. 5. Paired laggards at Anaphase (0.4%) (X 1500)

Fig. 6. Multiple bridges at Anaphase (0.8%) (X 1500)

Fig. 7. Equal separation of Chromatids at Anaphase (0.4%) (X 1500)

Fig. 8. 1 III + 5 II's + 9 I's at Metaphase-I (0.6%) (X 1500)

Fig. 9. 10 II's + 2 I's at Metaphase-I (0.4%) (X 1500)

Fig. 10. 9 II's + 4 I's at Metaphase-I (0.6%) (X 1500)

Fig. 11. 11 II's at Metaphase, 5 bivalents showing precocious separation (X 1500)

Fig. 12. 11 bivalents at Metaphase-I, showing 11 bivalent (0.4%) (X 1500)

PLATE - 32



2

5

4

6

8

9

7



12

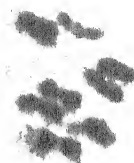


PLATE - 33 (Effect of EMS on A. albicans; Meiosis)

Fig. 13. 11 II's at Metaphase-I (0.2%) (X 1500)

Fig. 14. delayed separation of one bivalent at Anaphase-I (0.6%) (X 1500)

Fig. 15. delayed separation of one bivalent at Anaphase-I (0.4%) (X 1500)

Fig. 16. Two univalents away from the Anaphasic group (0.4%) (X 1500)

Fig. 17. Laddering chain of 4 chromosomes at late Anaphase-I (X 1500)

Fig. 18. Only two daughter nuclei at telophase-II, 3 chromatids lying in the cytoplasm (X 1500)

Fig. 19. Triad and micronuclei (0.6%) (X 600)

Fig. 20. Regular tetrads and dyad with 2 micronuclei (X 600)

Fig. 21. Pollen grains showing variation in size of fertile pollen grains (X 600)

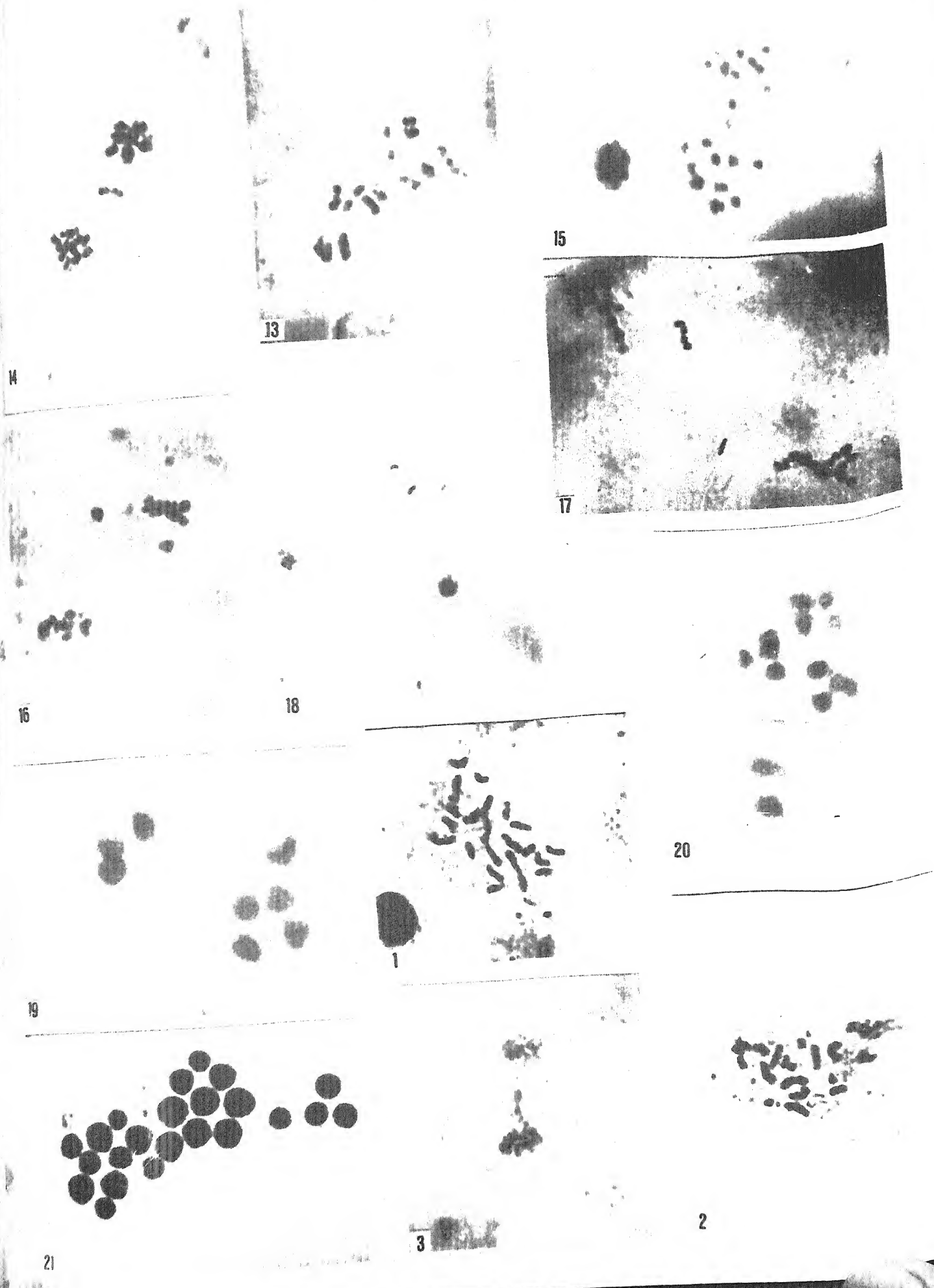
Fig. 1-3: (Effect of EMS on D. scarabi; Mitosis)

Fig. 1. chain of 4 sticky chromosomes (0.4%) (X 1500)

Fig. 2. Chromosome breakage (0.8%) (X 1500)

Fig. 3. Multiple bridges at Anaphase (0.8%) (X 1500)

PLATE - 33



traid and tetrad formation was observed. Pollen fertility percentage was 80.0.

Effect of EMS on seed germination and plant survival in A. scarabaeoides.

Gradual reduction in the percentage of seed germination and plant survival was noticed with increasing concentration and duration of treatments (Table-171). Observations on seed germination in petridishes, emergence of plumules in field and survival to maturity in 4 and 8 hours treatments at different concentrations are as follows:

4 hours treatments

After the treatment with the lowest concentration of (0.2%) EMS solution for 4 hours, seed germination, plumule emergence and survival to maturity were 92.0, 86.9 and 100.0 per cent respectively. At 0.4% concentration, 80.0 per cent seed germination, 85.0 per cent plumule emergence and 88.8 per cent survival to maturity was recorded. In the treatment with 0.6 and 0.8 per cent solutions gradual reduction in the percentage of seed germination, plumule emergence and survival to maturity was noticed (Table-171). When the highest concentration of 1.0% EMS solution was used, only 16.0 per cent seeds could germinate, and perhaps plumules could not emerge due to toxic effects of the chemical. Thus, no seedling could be raised after such a treatment.

8 hours treatment:

After the treatment with 0.2% EMS solution, 88.0 per cent seeds germinated, 86.3 per cent plumules emerged

traid and tetrad formation was observed. Pollen fertility percentage was 80.0.

Effect of EMS on seed germination and plant survival in
A. scarabaeoides.

Gradual reduction in the percentage of seed germination and plant survival was noticed with increasing concentration and duration of treatments (Table-171). Observations on seed germination in petridishes, emergence of plumules in field and survival to maturity in 4 and 8 hours treatments at different concentrations are as follows:

4 hours treatments

After the treatment with the lowest concentration of (0.2%) EMS solution for 4 hours, seed germination, plumule emergence and survival to maturity were 92.0, 86.9 and 100.0 per cent respectively. At 0.4% concentration, 80.0 per cent seed germination, 85.0 per cent plumule emergence and 88.8 per cent survival to maturity was recorded. In the treatment with 0.6 and 0.8 per cent solutions gradual reduction in the percentage of seed germination, plumule emergence and survival to maturity was noticed (Table-171). When the highest concentration of 1.0% EMS solution was used, only 16.0 per cent seeds could germinate, and perhaps plumules could not emerge due to toxic effects of the chemical. Thus, no seedling could be raised after such a treatment.

8 hours treatment:

After the treatment with 0.2% EMS solution, 88.0 per cent seeds germinated, 86.3 per cent plumules emerged

and 97.5 per cent plants survived to maturity (Table-171). 0.4% solution when used for 8 hours, percentage seed germination reduced to 72.0, plumule emergence 83.0 and plant survival 86.6. In the treatment with 0.6% and 0.8% EMS solutions, seed germination percentage was recorded as 60.0 and 44.0 respectively. A subsequent reduction in plumule emergence the field and plant survival to maturity was noticed (Table-171). At the highest concentration of EMS solution (1.0%), only 12.0 per cent of seeds could germinate. The plumules could not emerge after such a treatment.

Morphological observations in EMS treated plants of *A. scarabaeoides*.

Morphological observations in control, M_1 and M_2 plants of *A. scarabaeoides* are summarised in Table-172. Details of observations, at each concentration and duration of EMS treatments are as follows.

4 hours treatment:

0.2 % :

M_1 plants showed 34.0 cm average spread and 6.3 and 8.2 average primary and secondary branches respectively. Average length and breadth of central leaflet was 2.9 cm and 1.5 cm respectively. Days to 50% flowering and maturity were nearer to those of control plants. Average number of pods per plant was 28.0 and seeds per pod 2.5.

In M_2 plants, average spread of plants was 36.0 cm, Number of primary and secondary branches were 6.5 and 9.2 respectively. Length and breadth of central leaflet was 3.0 cm and 1.7 cm respectively. Days to 50% flowering and maturity were nearer to those of control plants. On an

average, number of pods per plant and seeds per pod were 34.0 and 2.6 respectively.

0.4 % :

M_1 plants showed reduction in plant spread, number of primary and secondary branches, pods per plant and seeds per pod (Table-172). In M_2 increase over M_1 , in these characters were recorded.

0.6%

In M_1 plants average plant spread was 28.0 cm. Number of primary and secondary branches were 5.3 and 7.5 respectively. On an average, length and breadth of central leaflet were 2.6 cm and 1.4 cm respectively. Days to 50% flowering and maturity were nearer to those of control. Average number of pods per plant and seeds per pod were 15.0 and 1.6 respectively.

M_2 plants showed 38.0 cm average plant spread and number of primary and secondary branches 5.8 and 8.6 respectively. Days to 50% flowering and maturity were nearer to those of control plants. Average number of pods per plant was 28.0 and seeds per pod 1.8.

1.8 %:

M_1 plants showed 25.0 cm average spread. Number of primary and secondary branches were 5.0 and 7.0 respectively. Average central leaf length and breadth were 2.5 and 1.4 cm² respectively. Days to 50% flowering and maturity were nearer to those of control. On an average number of pods per plant and number of seeds per pod were 6.0 and 1.1 respectively.

In M_2 plants, 35.0 cm, average plant spread was recorded. Number of primary and secondary branches were 5.6 and 7.5 respectively. Average central leaflet length and breadth were 2.7 and 1.4 cm respectively. Days to 50 % flowering and maturity were nearer to those of control. On an average the number of pods per plant and seeds per

pod were 25 and 1.7 respectively.

8 hours treatment:

0.2 % :

M_1 plants showed 25.0 cm average spread. Number of primary and secondary branches were 6.1 and 8.1 respectively. Plants showed 2.8 cm average central leaflet length and 1.5 cm breadth. Days to 50% flowering and maturity were 98 and 153 respectively. On an average number of pods per plant and seeds per pod were 25 and 2.0 respectively.

In M_2 plants, average plant spread was 39.0 with 6.2 primary and 8.2 secondary branches. Central leaf length and breadth were 2.6 and 1.6 cm respectively. Days to 50% flowering and maturity were nearer to those of control plants (Table-192). On an average, number of pods per plant and seeds per pod were 30 and 2.4 respectively.

0.4 % :

Average plant spread in M_1 plants was 31.0 cm. Number of primary and secondary branches were 6.0 and 8.0 respectively. Plants showed average 2.0 cm central leaflet length and 1.6 cm breadth. Days to 50% flowering and maturity were nearer to those of control plants. On an average, the number of pods per plant was 20.0 and seeds per pod 2.0.

M_2 plants showed 40.0 cm average plant spread. Number of primary and secondary branches were 6.1 and 8.2 respectively. Plants showed 2.6 cm average leaflet length and 1.5 cm breadth. Days to 50% flowering and maturity were nearer to those of control plants. On an average, the

number of pods per plant and seeds per pod were 28.0 and 2.1 respectively.

0.6 % :

M_1 plants showed 29.0 cm average plant spread. Number of primary and secondary branches were 5.3 and 8.0 respectively. Days to 50% flowering and maturity were nearer to those of control plants. On an average, number of pods per plant was 12.0 and seeds per pod 1.5.

In M_2 plants, 40.0 cm average plant spread was recorded. Average number of primary and secondary branches were 6.0 and 8.4 respectively. Plants showed 2.2 cm average central leaflet length and 1.5 cm breadth. Days to 50% flowering and maturity were nearer to those of control plants. Average number of pods per plant and seeds per pod were 26 and 1.6 respectively.

0.8 %:

M_1 plants showed 31.0 cm average plant spread. Number of primary and secondary branches were 4.0 and 6.8 respectively. Plants showed 2.1 cm average leaf length and 1.3 cm breadth. Days to 50% flowering and maturity were nearer to those of control plants. On an average number of pods per plant was 5.0 and seeds per pod 1.0.

In M_2 plants, average plants spread was 38.0 cm and number of primary and secondary branches were 5.1 and 7.1 respectively. Average central leaf let length was 2.3 cm and breadth 1.4 cm at this concentration and duration. Days to 50% flowering and maturity were nearer to those of control plants. On an average number of pods per plant was 20.0 and seeds per pod was 1.3.

Cytology (M₁ plants)Mitosis:

Mitotic study made in the root tip cells of M₁ seeds revealed chromosome stickiness, clumping and breakage at metaphase (Plate-33). Chromosome breakage was more prevalent and increased with increase in the concentration of the chemical (Table-173). Observations detectable at somatic metaphase are as follows:

4 hours treatment:

Even at the lowest concentration (0.2%), chromosome stickiness (Plate-33; Fig. 1) and clumping was observed in 4.0 per cent of cells (Table-173). 0.4% EMS used for 4 hours revealed chromosome breakage clumping and stickiness in 4.0, 4.0 and 6.0 per cent metaphase cells respectively. At anaphase chromatid bridge was observed in 4.0 per cent cells. At 0.6% concentration, increase in percentage of cells showing chromosome breakage was observed (Table-173). At the highest concentration of EMS used for 4 hours, 30.0 per cent cells revealed chromosome breakage and 12.0 per cent clumped chromosomes at metaphase. At ^{0.8}% concentration, anaphase bridge without fragments and also with fragments were observed in 8.0 and 4.0 per cent cells respectively.

8 hours treatment:

At 0.2% concentration normal somatic chromosomes were noticed except in 6.6 per cent cells where sticky chromosomes were recorded (Table-173). At anaphase equal separation of chromatids was observed. At 0.4% concentration chromosome break was observed in 4.0 per cent cells. At anaphase bridge with fragments and without fragment were

observed in 4.0 and 4.0 per cent cells respectively. When 0.6% EMS solution used for 8 hours, a subsequent increase in chromosome breakage (Plate-33; Fig. 2) was recorded (Table-173). At anaphase somatic bridge (Plate-33; Fig. 3) with fragment and without fragment was observed in 8.0 and 12.0 per cent cells respectively. The highest concentration of 9.8 % EMS solution showed 32.0 per cent cells with chromosome break, 12.0 per cent cells sticky chromosomes and 16.0 per cent cells clumped chromosomes. At anaphase, bridge with fragment and without fragment were observed in 12.0 and 16.0 per cent cells respectively (Table-173).

Meiosis (M_1 plants):

Meiotic study of M_1 plants revealed association of 6, 4, 3 and 2 chromosomes at metaphase-I (Table-174) and Plate-34). Gradual increase in the frequency of trivalents and rod bivalents at metaphase-I and delayed separation of bivalent and laggards at anaphase-I, with increasing concentration was recorded. Observations at each concentration and duration of treatments are as follows.

4 hours treatment:

0.2 %:

At Metaphase-I, ring bivalents ranged from 3-11 with 8.84 per cell and rod bivalents from 0-8 with 2.00 per cell. Univalents ranged from 0-2 with 0.22 per cell. At anaphase-I, delayed separation of one bivalent was observed in 2.22 per cent cells. At anaphase-II, normal separation was observed resulting in regular tetrad formation and high pollen fertility (91.54%).

0.4 %:

At metaphase-I, trivalents ranged from 0-1 with 0.02 per cell. Ring and rod bivalents ranged from 3-11 and 0-8 with 2.00 and 8.47 per cell respectively. Univalents ranged from 0-2 with 0.95 per cell. At anaphase-I, delayed separation of bivalent and formation of laggards were observed in 1.92 and 1.92 per cent cells. At anaphase-II laggards were observed in 3.84 per cent cells. At sporad stage regular tetrad formation was observed. Pollen fertility was 78.24 per cent.

0.6 %:

At metaphase-I, hexavalent ranged from 0-1 with 0.02 per cell and quadrivalents from 0-1 with 0.14 per cell. A range of 0-2 trivalents (Plate-34; Fig. 4) was observed with 0.09 per cell (Table-174). Ring and rod bivalents ranged from 0-11 and 0-11 with 1.95 and 10.68 per cell respectively. Univalents ranged from 0-4 with 0.58 per cell. At anaphase-I and II laggards were observed in 5.12 and 2.56 per cent cells respectively. At sporad stage, normal tetrad formation was observed except in some cells where micronuclei were recorded. Pollen fertility was 52.61 per cent.

0.8 %:

At metaphase-I, hexavalent and quadrivalent (Plate-34; Fig. 5, 8) ranged from 0-2 and 0-1 with 0.06 and 0.18 per cell respectively. Trivalents and univalents ranged from 0-2 and 0-4 with 0.09 and 0.39 per cell respectively. Ring bivalents ranged from 0-11 with 1.54 per cell and rod bivalents ranged from 0-1 with 10.0 per cell. At anaphase-I and II laggards (Plate-34; Fig. 13) were noticed in 3.22 and 4.83 per cent cells respectively. At sporad stage, tetrads and micronuclei were also recorded. Pollen fertility was 37.31 per cent.

8 hours treatment:0.2 % :

At metaphase-I, ring bivalents ranged from 3-11 with 8.90 per cell and rod bivalents ranged from 0-8 with 1.98 per cell. Univalents ranged from 0-2 with 0.22 per cell. At anaphase-I and II equal separation of chromosomes to the poles was observed. Regular tetrad formation was observed resulting in 90.85 per cent pollen fertility.

0.4 % :

At metaphase-I, trivalents ranged from 0-1 with 0.01 per cell. Ring and rod bivalents ranged from 3-11 and 0-8 with 2.66 and 8.00 per cell respectively. Univalents ranged from 0-2 with 0.62 per cell. At anaphase-I, delayed separation of one bivalent was observed in 2.0 per cent cells. At anaphase-II equal separation of chromosomes to the poles was observed in all the cells studied. Regular tetrad formation was seen at spored stage. Pollen fertility was 75.11 per cent.

0.6 %:

At metaphase-I, hexavalent and quadrivalent (Plate-34; Fig. 9) ranged from 0-1 and 0-1 with 0.02 and 0.08 per cell respectively. At this stage, trivalents and univalents ranged from 0-1 and 0-4 with 0.04 and 0.43 per cell respectively. Ring and rod bivalents ranged from 0-11 and 0-11 with 2.00 and 8.47 per cell. At anaphase-I, delayed separation of bivalent and formation of laggards were observed in 2.0 and 4.0 per cent cells respectively. At anaphase-II laggards were noticed in 1.90 per cent cells. At sporad stage regular tetrad formation was observed except in some cells where one to two micronuclei were

recorded. Pollen fertility was 51.66 per cent.

0.8 %:

At metaphase-I, hexavalent and quadrivalents ranged from 0-1 and 0-1 with 0.03 and 0.20 per cell respectively. Trivalents (Plate-34; Fig. 10) and univalents ranged from 0-2 and 0-4 with 0.03 and 0.065 per cell respectively. At anaphase-I, delayed separation, laggards and chromatid bridge (Plate-34; Fig. 12) were observed in 6.0, 4.0 and 2.0 per cent cells respectively. At anaphase-II, laggard were observed in 6.00 per cent cells. At spored stage, tetrads and micronuclei (Plate-34; Fig. 14) were observed and percentage pollen fertility was 40.0 (Table-174).

Effect of EMS on seed germination and plant survival in *Cajanus cajan* (ICP 8647).

Observations on seed germination in petridishes, emergence of plumules in the field and survival to maturity in 4 and 8 hours EMS treatments at different concentrations and durations are as follows:

4 hours treatment:

After treatment with the lowest concentration (0.2%) of EMS solution 86.0% seeds germinated, 76.2% plumules emerged and 80.4 % plants survived to maturity. At higher concentration (0.4 and 0.6%) further decreased in seed germination, plumule emergence and plant survival was noticed (Table-175). At 0.8% concentration, seed germination and plumule emergence were 30.0 and 3.22 per cent, but no seedling could survive after this treatment. In the treatment with the highest concentration of EMS

Table - 171

Germination of EMS treated seeds of Atylosia scarabaeoides
(No. of seeds treated in each case was 25).

| Concen- tration (%) | Duration of treat- ment (hours) | Germination in petridish (%) | Emergence of plumule in field (%) | Survival to matu- rity (%) |
|---------------------------|--|------------------------------------|--|----------------------------------|
| Control | - | 96.0 | 95.8 | 100 |
| 0.2 | 4 | 92.00 | 86.9 | 100 |
| " | 8 | 88.0 | 86.3 | 97.5 |
| 0.4 | 4 | 80.0 | 85.0 | 88.8 |
| " | 8 | 72.0 | 83.3 | 86.6 |
| 0.6 | 4 | 64.0 | 80.6 | 84.6 |
| " | 8 | 60.0 | 73.3 | 81.8 |
| 0.8 | 4 | 52.0 | 69.2 | 66.6 |
| " | 8 | 44.0 | 63.6 | 57.1 |
| 1.0 | 4 | 16.0 | NIL | - |
| " | 8 | 12.0 | NIL | - |

Morphological observations in control, M₁ and M₂ plants of Atylosia scarabaeoides.

| Characters | Control | Gene- ration | 4 hours treatments | | | | 8 hours treatments | | | |
|-------------------------------|---------|-----------------|--------------------|---------|---------|---------|--------------------|---------|---------|---------|
| | | | 0.2% | 0.4% | 0.6% | 0.8% | 0.2% | 0.4% | 0.6% | 0.8% |
| Spread of plant (cm) | 40.1 | M ₁ | 34 | 30 | 28 | 25 | 33 | 31 | 29 | 31 |
| | 37.2 | M ₂ | 36 | 36 | 38 | 35 | 39 | 40 | 40 | 38 |
| No. of primary branches | 6.6 | M ₁ | 6.3 | 5.4 | 5.3 | 5.0 | 6.1 | 6.0 | 5.3 | 4.0 |
| | 5.1 | M ₂ | 6.5 | 6.4 | 5.8 | 5.6 | 6.2 | 6.1 | 6.0 | 5.11 |
| No. of Secondary branches | 9.1 | M ₁ | 8.2 | 8.0 | 7.5 | 7.0 | 8.1 | 8.0 | 8.0 | 6.8 |
| | 8.6 | M ₂ | 9.2 | 8.5 | 8.6 | 7.5 | 8.2 | 8.2 | 8.4 | 7.1 |
| Central leaflet (L x B) cm | 2.7x1.5 | M ₁ | 2.9x1.5 | 2.8x1.4 | 2.6x1.4 | 2.5x1.6 | 2.8x1.5 | 2.0x1.6 | 2.1x1.6 | 2.1x1.3 |
| | 2.9x1.7 | M ₂ | 3.0x1.7 | 3.0x1.6 | 2.8x1.6 | 2.7x1.4 | 2.6x1.6 | 2.6x1.5 | 2.2x1.5 | 2.3x1.4 |
| Days to flowering | 97 | M ₁ | 97 | 99 | 99 | 100 | 98 | 98 | 99 | 99 |
| | 100 | M ₂ | 99 | 100 | 101 | 101 | 99 | 99 | 100 | 100 |
| Days to maturity | 152 | M ₁ | 150 | 151 | 151 | 153 | 153 | 151 | 150 | 152 |
| | 153 | M ₂ | 151 | 153 | 152 | 155 | 152 | 151 | 152 | 152 |
| Pods per plant | 26.5 | M ₁ | 28 | 25 | 15 | 6 | 25 | 20 | 12 | 5 |
| | 30.2 | M ₂ | 34 | 32 | 28 | 25 | 30 | 28 | 26 | 20 |
| Seeds per pod | 2.6 | M ₁ | 2.5 | 2.1 | 1.6 | 1.1 | 2.2 | 2.0 | 1.5 | 1.0 |
| | 2.4 | M ₂ | 2.6 | 2.2 | 1.8 | 1.7 | 2.4 | 2.1 | 1.6 | 1.3 |

* In each generation 10 plants were studied

Table - 173
Mitotic observations in Atylosia scarabaeoides (M₁)

| Concentration (%) | Duration (hrs.) | METAPHASE | | | | ANAPHASE | | | |
|-------------------|-----------------|----------------------|---|------------|----------|----------------------|-------------------|----------|-------------------|
| | | No. of cells studied | Uneffect- ed cell some breakage (2n = 22) | Stickiness | Clumping | No. of cells studied | Normal separation | Bridge | Bridge + fragment |
| Control | - | 25 | 25 (100) | - | - | 25 | 25 (100) | - | - |
| 0.2 | 4 | 25 | 23 (92.0) | 1 (4.0) | 1 (4.0) | 25 | 25 (100) | - | - |
| 0.2 | 8 | 30 | 20 (66.6) | 2 (6.6) | 1 (3.3) | 25 | 25 (100) | - | - |
| 0.4 | 4 | 50 | 43 (86.0) | 2 (4.0) | 3 (6.0) | 25 | 24 (96.0) | 1 (4.0) | - |
| 0.4 | 8 | 25 | 21 (84.0) | 1 (4.0) | 2 (8.0) | 25 | 23 (92.0) | 1 (4.00) | 1 (4.00) |
| 0.6 | 4 | 50 | 38 (76.0) | 5 (10.0) | 4 (8.0) | 25 | 23 (92.0) | 1 (4.00) | 2 (8.00) |
| 0.6 | 8 | 50 | 39 (78.0) | 6 (12.0) | 4 (8.0) | 25 | 20 (80.0) | 3 (12.0) | 2 (8.0) |
| 0.8 | 4 | 50 | 24 (48.0) | 15 (30.0) | 5 (10.0) | 25 | 22 (88.0) | 2 (8.0) | 1 (4.0) |
| 0.8 | 8 | 50 | 20 (40.0) | 16 (32.0) | 6 (12.0) | 25 | 18 (72.0) | 4 (16.0) | 3 (12.0) |

(Figures in parentheses are per cent.)

Table - 174

Melotic observation in M_1 plants of Atylosia scarabaeoides. (No. of plants studied in each case were 5)

were 5)

Concen-
tration
(%)

Dura-
tion
(%)

No. of
cells
studi-
ed

Chromosomal associations at M-I

VI

IV

III

Ring
II

Red
II

I

Anaphase - I

No. of Delay-
ed
cells
studi-
ed

ed
(%)

Lag-
gs.
(%)

Brid-
ge
(%)

No. of
cells
studi-
ed

ed
(%)

Lag-
gs.
(%)

Pollen
ferti-
lity
(%)

Control

-

30

-

-

-

10-11
(10.8)

0-1
(0.2)

-

50

-

-

-

40

-

99.4

0.2

4

50

-

-

-

3-11
(8.84)

0-8
(2.00)

0-2
(0.22)

45

2.22

-

-

36

-

91.54

"

8

45

-

-

-

3-11
(8.90)

0-8
(1.98)

0-2
(0.22)

50

2.00

-

-

43

-

90.85

0.4

4

46

-

-

0-1
(.02)

3-11
(2.00)

0-8
(8.47)

0-2
(0.95)

52

1.92

3.84

-

52

1.92

78.24

"

8

53

-

-

0-1
(.01)

3-11
(2.66)

0-8
(8.00)

0-2
(0.62)

50

2.00

2.99

-

45

-

75.11

0.6

4

41

0-1
(.02)

0-1
(.14)

0-2
(.09)

0-11
(1.95)

0-11
(10.88)

0-4
(0.58)

39

2.56

5.12

-

40

2.56

52.61

"

8

46

0-1
(.02)

0-1
(.08)

0-2
(.04)

0-11
(2.00)

0-11
(8.47)

0-4
(0.43)

50

2.00

4.00

-

51

1.90

51.66

0.8

4

61

0-2
(.06)

0-1
(.18)

0-2
(.09)

0-11
(1.54)

0-11
(10.00)

0-4
(.39)

62

3.22

4.83

3.22

60

4.83

37.81

"

8

52

0-1
(.03)

0-1
(.20)

0-2
(.03)

0-11
(1.55)

0-11
(3.37)

0-4
(.55)

50

6.00

4.00

2.00

50

6.00

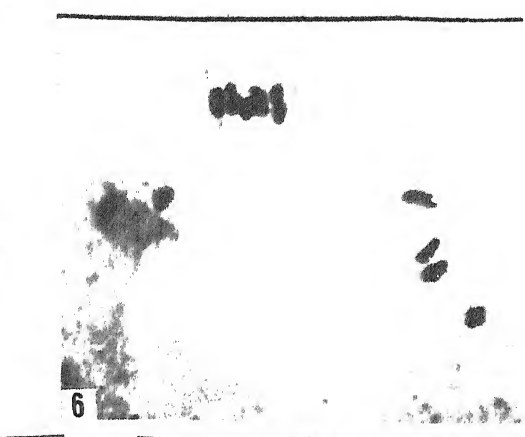
40.00

(Mean values in parentheses)

PLATE - 34 (Effect of EMS on A. scarab. : Meiosis)

- Fig. 4. 2 III's + 8 II's at Metaphase -I (X 1500)
- Fig. 5. 1 IV + 8 II's + 2 I's at Metaphase-I (0.4%)
(X 1500)
- Fig. 6. 11 bivalent at Metaphase-I showing multipolar
- Fig. 7. 2 IV's + 6 II's + 2 I's (one dividing unival
at Metaphase-I (0.6%) (X 1500)
- Fig. 8. 1 VI + 8 II's at Metaphase-I (0.8%) (X 1500)
- Fig. 9. 1 IV + 9 II's at Metaphase-I (0.6%) (X 1500)
- Fig. 10. 1 IV + 2 III's + 6 II's at Metaphase-I (0.8%)
(X 1500)
- Fig. 11. Delayed separation of two bivalents at
Anaphase-I (0.8%) (X 1500)
- Fig. 12. Chromatid bridge at Anaphase-I (0.8%) (X 1500)
- Fig. 13. 3 Chromatids away from the groups at
Anaphase-II (X 1500)
- Fig. 14. Two micronuclei with normal tetrad (0.8%)
(X 600)
- Fig. 15. Pollen grains showing sticky groups and size
variation (X 600)

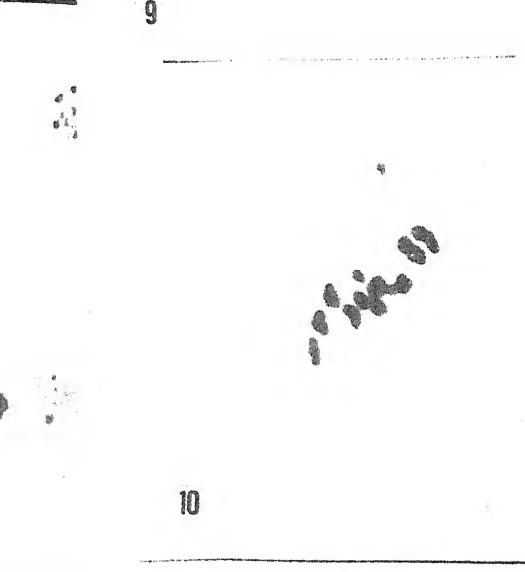
... : ...
 ... (x 100)
 ... (0.4)
 ... multiple
 ... multiplying with
 ...
 ... (x 100)
 ... (x 100)
 ... (0.4)
 ... :
 ... (x 100)
 ... at
 ... (0.4)
 ... and size



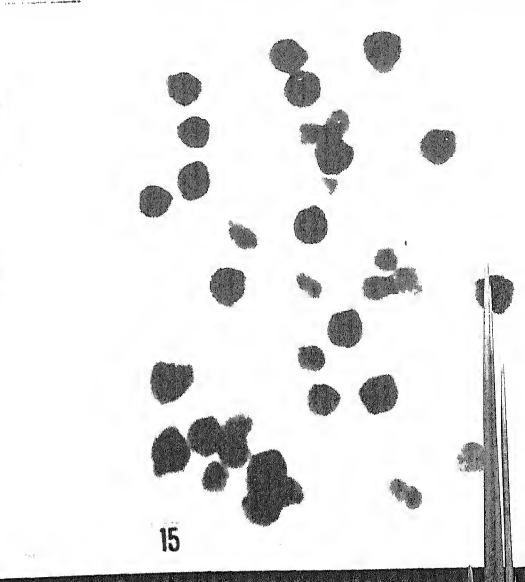
6



3



10



15

PLATE - 34

5

6

8

9

7

12

10

11

13

14

15

solution (1.0%) plumules could not emerge though 4.0 per cent seeds germinated.

8 hours treatment:

Percentage seed germination, plumule emergence and plant survival till maturity were slightly reduced at the lowest concentration (0.2%) as compared to 4 hours treatment (Table-175). At 0.4%, 80.0 per cent seeds germinated, 71.25 per cent plumules emerged and 81.70 per cent plants survived. At 0.6% concentration, further decrease in seed germination, plumule emergence and plant survival was noticed (Table-175). Treatment with 0.8% concentration revealed 30.0% seed germination and 32.2 plumule emergence but seedlings could not survive after such a treatment. At the highest concentration (1.0%) only 2.0 per cent seeds could germinate.

Thus, reduction percentage of seed germination, plumule emergence and plant survival to maturity was linear with increase in concentration.

Morphological observations in EMS treated plants of *Cajanus cajan*. (F CP 8647)

Morphological observations in control, M_1 and M_2 plants of *Cajanus cajan* are summarised in Table-176. The details are as follows:

4 hour treatment:

0.2 % :

M_1 plants had 169.0 cm average height, 6.1 primary and 16.8 secondary branches. Length of central leaflet was 5.5 cm and breadth 1.7 cm. Days to 50% flowering and

maturity were nearer to those of control plants. Pods per plant was 80.0 and seeds per pod 2.6.

M_2 plants showed 172.0 cm average height, 7.8 primary and 22.2 secondary branches. Days to 50% flowering and maturity were nearer to those of control plants. Pods per plant was 89.0 and seeds per pod 2.8.

0.4 %:

M_1 plants showed 163.0 cm average height, 5.5 primary and 15.2 secondary branches. A slight reduction in central leaflet length was observed as compared to control plants (Table-176). Other than trifoliate leaves, bifoliate and quadrifoliate leaves were also observed. Days to 50% flowering and maturity were slightly delayed (Table-176). Pods per plant was 72.1 and seeds per pod 2.1.

M_2 plants showed 174.0 cm average height, 6.8 primary and 17.5 secondary branches. Length of central leaflet was 5.4 cm and breadth 1.6 cm. Days to 50% flowering and maturity were nearer to those of control plants. Pods per plant was 82.5 and seeds per pod 2.6.

0.6 %:

M_1 plants had 161.0 cm average height, 2.9 primary and 11.8 secondary branches. Length and breadth of central leaflet were 5.11 and 1.7 respectively. Days to 50% flowering and maturity were delayed by 4 and 8 days respectively. Pods per plant was 60.1 and seeds per pod 1.6.

M_2 plants showed 171.0 cm average height, 3.1 primary and 17.1 secondary branches. Days to 50% flowering and maturity were nearer to those of control plants. Pods per plant and seeds per pod were increased over M_1 plants (Table-176).

8 hours treatment:0.2 %:

M₁ plants had nearly similar height, number of primary and secondary branches to those of control (Table-176). Length of central leaflet was 5.4 cm and breadth 1.6 cm. Days to 50% flowering and maturity were nearer to those of control. Pods per plant was 82.0 and seeds per pod 2.5.

M₂ plants showed an increase in average height, number of primary and secondary branches, length and breadth of central leaflet, pods per plant and seeds per pod, over M₁ plants (Table-176).

0.4 %:

M₁ plants had 163.0 cm average height, 5.0 primary and 12.2 secondary branches. Length of central leaflet was 5.3 cm and breadth 1.7 cm. Days to flowering and maturity were delayed by 3 days. Pods per plant was 71.5 and seeds per pod 2.0.

M₂ plants showed 171.0 cm average height, 7.5 primary and 15.5 secondary branches. Length and breadth of central leaflet were 5.5 cm and 1.6 cm respectively. Days to 50% flowering and maturity were nearer to those of control plants. Pods per plant was 79.2 and seeds per pod 2.1.

0.6 %:

M₁ plants showed reduction in height, number of primary and secondary branches (Table-176). Length and breadth of central leaflet were 5.0 cm and 1.5 cm

respectively. Days to 50% flowering and maturity were delayed by 5 and 9 days. Pods per plant was 56.0 and seeds per pod 1.4.

M₂ plants had 170.0 cm height, 6.2 primary and 15.2 secondary branches. Days to 50% flowering and maturity were nearer to those of control plants. Average number of pods per plant was 75.0 and seeds per pod 2.0.

Cytology:

Mitosis:

Chromosomal abnormalities studied during mitotic divisions (Plate-35) are summarised in Table-177. Their details are as follows:

4 hour treatment:

At 0.2% concentration, no cytological abnormality was noticed. At 0.4% concentration, chromosome stickiness, clumping and breakage (Fig. 2,3) was noticed in 2.0, 6.0 and 4.0 per cent of cells at metaphase respectively. At anaphase bridge (Plate-35; Fig. 4) and bridge + fragments (Plate-35; Fig. 5) were observed in 4.0 and 2.0 per cent cells respectively. The treatment with 0.6% EMS exhibited further increase in percentage of cells showing chromosome stickiness clumping and breakage (Table-177). The highest concentration of 0.8% EMS solution revealed chromosome breakage in 16.0% cells. At anaphase bridge with and without fragments were recorded in 4.0 and 8.0 per cent of cells respectively. In 4.0 per cent cells lagging fragments (Plate-35; Fig. 7) were noticed.

8 hours treatment:

Mitosis followed the normal course after the treatment with 0.2% EMS solution. At 0.4% concentration

chromosome stickiness, clumping and breakage was noticed in 6.0, 4.0 and 6.0 per cent cells respectively. At 0.6% concentration further increase in chromosome anomalies at metaphase and anaphase was recorded (Table-177). At the highest concentration (0.8%), chromosome breakage (Plate-35; Fig. 4) was recorded in 20.0 per cent of cells and stickiness and clumping were shown by 20.0 and 8.0 per cent cells respectively. Remaining 52.0 per cent cells showed normal mitotic chromosomes. At anaphase increase in the percentage of cells showing bridge with and without fragments was observed (Table-177).

Meiosis (M_1 plants):

Observations on chromosomal configurations (Plate-35;36) at each concentration and duration of treatments (Table-178) are as follows:

4 hours treatment:

0.2% :

No meiotic abnormality was recorded except occurrence of two univalents (in 0.16 per cells). Pollen fertility was 91.81 per cent.

0.4 %:

Multivalents viz., quadrivalents (Plate-35; Fig.9) and trivalents (Plate-35; Fig. 11) were observed at metaphase-I with the frequency of 0.04 and 0.06 per cell respectively. Ring bivalents ranged from 5-11 with 4.51 per cell and rod bivalents ranged from 0-6 with 5.97 per cell. At metaphase-I, univalents ranged from 0-2 with 0.28 per cell. Laggarde at anaphase-I (Plate-36; Fig. 16) and II (Plate-36; Fig. 20) were observed in 6.89 and 2.11 per

cent cells respectively. At sporad stage dyad, triad, tetrad and micronuclei (Plate-36; Fig. 22) were seen. Pollen fertility was 78.55 per cent (Table-178).

0.6 %:

At metaphase-I, higher chromosome association viz., hexavalent (Plate-35; Fig. 10) was also observed with the frequency of 0.05 per cell. Quadrivalents and trivalents ranged from 0-1 and 0-1 with 0.14 and 0.17 per cell respectively. Frequency of ring and rod bivalents were 1.94 and 8.25 respectively. Univalents at metaphase-I ranged from 0-2 and 0.1% per cell. Grouping of bivalents into 2-4 groups was observed frequently at M-1 (Plate-36; Fig. 14). At anaphase-I and II, laggards were recorded in 10.89 and 5.0 per cent cells respectively. At sporad stage, dyad, triad, tetrad and micronuclei were seen. Pollen fertility was 51.82 per cent.

8 hours treatment:

0.2%:

Meiosis followed normal course, except occasional occurrence of 1-2 univalents. Pollen fertility was 90.70 per cent.

0.4 %:

At metaphase-I frequency of multivalents viz., quadrivalents and trivalents were 0.06 and 0.02 per cell respectively. Ring bivalents ranged from 5-11 and rod bivalents from 0-6. Univalents ranged from 0-2 with 0.22 per cell. At anaphase-I and II, laggards were observed in 6.0 and 2.0 per cent cells respectively. At sporad stage other than tetrads, micronuclei were also seen. Pollen fertility was 75.80%.

Table - 175

Germination of EMS treated seeds of Cajanus cajan
(ICP 8647). (In each case No. of seeds treated was 50)

| Concen- tration of EMS (%) | Duration of treat- ment (hours) | Germination in petridishes (%) | Emergence of seedl- ings in field (%) | Survival to maturi- ty (%) |
|-------------------------------------|--|--------------------------------------|---|----------------------------------|
| Control | - | 98.6 | 91.83 | 96.66 |
| 0.2 | 4 | 86.0 | 76.22 | 80.40 |
| " | 8 | 84.0 | 74.11 | 79.36 |
| 0.4 | 4 | 82.0 | 73.17 | 83.33 |
| " | 8 | 80.0 | 71.25 | 80.70 |
| 0.6 | 4 | 54.0 | 69.81 | 81.08 |
| " | 8 | 50.0 | 68.62 | 78.37 |
| 0.8 | 4 | 30.0 | 3.22 | NIL |
| " | 8 | 22.0 | 4.54 | NIL |
| 1.0 | 4 | 4.0 | NIL | - |
| " | 8 | 2.0 | NIL | - |

Morphological observations in control, M₁ and M₂ plants of cajani (I CP 8607)

| Characters | Control | Gene- ration | 4 hours treatment | | | | 8 hours treatment | | | |
|--------------------------------|--------------------------|--|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|-------|-------|
| | | | 0.2 % | 0.4 % | 0.6 % | 0.2 % | 0.4 % | 0.6 % | 0.2 % | 0.6 % |
| Height of plant (cm) | 171 173 5.5 6.1 | M ₁ M ₂ M ₁ M ₂ | 169 172 6.1 7.8 | 165 174 5.5 6.8 | 161 171 2.8 3.1 | 170 175 6.0 8.0 | 163 171 5.0 7.5 | 160 170 3.5 6.2 | | |
| No. of primary | | | | | | | | | | |
| No. of secondary branches | 17.2 21.3 | M ₁ M ₂ | 16.8 22.2 | 15.2 17.5 | 11.8 17.1 | 15.2 18.3 | 12.2 16.5 | 10.1 15.2 | | |
| Central leaflet (L x B) cm. | 5.7x1.6 5.6x1.5 | M ₁ M ₂ | 5.5x1.7 5.6x1.7 | 5.3x1.6 5.4x1.6 | 5.1x1.7 5.3x1.6 | 5.4x1.6 5.4x1.5 | 5.3x1.7 5.5x1.6 | 5.0x1.5 5.2x1.6 | | |
| Days to flowering | 145 146 | M ₁ M ₂ | 144 145 | 147 145 | 149 145 | 145 145 | 148 144 | 150 145 | | |
| Days to maturity | 210 212 | M ₁ M ₂ | 208 212 | 214 211 | 218 213 | 210 211 | 213 215 | 219 217 | | |
| Pods per plant | 84.2 86.3 | M ₁ M ₂ | 80.0 89.0 | 72.1 82.5 | 60.1 79.5 | 82.0 86.0 | 71.5 79.2 | 56.0 75.0 | | |
| Seeds per pod | 2.8 2.6 | M ₁ M ₂ | 2.6 2.8 | 2.1 2.6 | 1.6 2.0 | 2.5 2.6 | 2.0 2.1 | 1.4 2.0 | | |

* In each generation no. of plants studied were = 5.

Table - 177

Mitotic observations in M_1 seeds of Cajanus cajan (ICP 8647) .

| METAPHASE | | | | ANAPHASE | | | |
|---------------------------|-------------------------|---|---------------------------|-----------------|---------------|---|--------------------------------|
| Concent- ration (%) | Dura- tion (hrs.) | No. of unef- fect- ed cells studied (2n = 222) | Chro- some breakage | Stick- iness | Clump- ing | No. of Normal cells separa- tion | Bridge + fragment laggs. |
| Control | - | 25 (100) | - | - | - | 25 (100) | - |
| 0.2 | 4 | 50 (98.0) | - | 1 (2.0) | - | 50 (100) | - |
| 0.2 | 8 | 25 (96.0) | - | 1 (4.0) | - | 30 (100) | - |
| 0.4 | 4 | 50 (88.0) | 2 (4.0) | 1 (2.0) | 3 (6.0) | 47 (94.0) | 1 (2.0) |
| 0.4 | 8 | 50 (84.0) | 3 (6.0) | 3 (6.0) | 2 (4.0) | 23 (92.0) | 1 (4.0) |
| 0.6 | 4 | 50 (70.0) | 5 (10.0) | 6 (12.0) | 4 (8.0) | 45 (90.0) | 2 (4.0) |
| 0.6 | 8 | 50 (66.0) | 6 (12.0) | 7 (14.0) | 4 (8.0) | 46 (92.0) | 1 (2.0) |
| 0.8 | 4 | 25 (60.0) | 4 (16.0) | 4 (16.0) | 2 (8.0) | 21 (84.0) | 1 (4.0) |
| 0.8 | 8 | 25 (52.0) | 5 (20.0) | 5 (20.0) | 2 (8.0) | 20 (80.0) | 2 (8.0) |

415

(figures in parentheses are per cent)

Table - 178

Meiotic observations in M_1 plants of Calanus calan (ICP 8647). No. of plants studied in each case were 5.

| Concen- tration (%) | Dura- tion (hrs.) | No. of cells studi- ed | Chromosome associations at M-I | | | | | | Anaphase - I | | Anaphase-II | | Pollen ferti- lity (%) |
|---------------------------|-------------------------|---------------------------------|--------------------------------|---------------|---------------|-----------------|---------------|---------------|----------------------------|--------------------|----------------------------|--------------|---------------------------------|
| | | | VI | IV | III | Ring II | Red II | I | No. of cells studied | Lag- gs. (%) | No. of cells studied | Lags. (%) | |
| Control | - | 30 | - | - | - | 10-11 (10.3) | 0-1 (0.60) | - | 50 | - | 80 | - | 99.34 |
| 0.2 | 4 | 48 | - | - | - | 9-11 (8.54) | 0-2 (2.08) | 0-2 (0.16) | 150 | 0.83 | 90 | - | 91.81 |
| 0.2 | 8 | 50 | - | - | - | 9-11 (8.64) | 0-2 (2.22) | 0-2 (0.2) | 100 | 2.00 | 85 | - | 90.70 |
| 0.4 | 4 | 47 | - | 0-1 (0.04) | 0-1 (0.06) | 5-11 (4.51) | 0-6 (5.97) | 0-2 (0.23) | 80 | 6.89 | 80 | 2.11 | 78.55 |
| 0.4 | 8 | 49 | - | 0-1 (0.06) | 0-1 (0.02) | 5-11 (4.71) | 0-6 (6.02) | 0-2 (0.22) | 50 | 6.00 | 50 | 2.0 | 75.80 |
| 0.6 | 4 | 35 | 0-1 (0.05) | 0-1 (0.14) | 0-1 (0.17) | 3-11 (1.94) | 0-8 (8.25) | 0-2 (0.17) | 45 | 10.79 | 45 | 5.00 | 51.82 |
| 0.6 | 8 | 38 | 0-1 (0.02) | 0-1 (0.05) | 0-1 (0.02) | 3-11 (3.61) | 0-8 (8.11) | 0-2 (0.05) | 50 | 12.00 | 50 | 6.0 | 52.50 |

(mean values in parentheses)

PLATE - 35 Effect of EMS on C. cajan (ICP 8647)

Fig. 2-8 : Mitosis; 9-13: Meiosis.

Fig. 1. Morphological variations in leaf shape and number.

Fig. 2. Chromosome breakage at Metaphase- (0.4%) (X 1500)

Fig. 3. Sticky fragments of Chromosomes (0.6%) (X 1500)

Fig. 4. Chromosome fragmentation at Metaphase (0.8%)
(X 1500)

Fig. 5. Multiple bridges at Anaphase (0.8%) (X 1500)

Fig. 6. Broken bridges at Anaphase (0.8%) (X 1500)

Fig. 7. Laggards at Anaphase- (0.8%) (X 1500)

Fig. 8. Chromosome showing stickiness at Metaphase
(0.4%) (X 1500)

Fig. 9. $1\text{IV} + 9\text{II}'\text{s}$ at Metaphase-I (0.4%) (X 1500)

Fig.10. $C_6 + 8\text{II}'\text{s}$ at Metaphase-I (0.6%) (X 1500)

Fig.11. $1\text{III} + 9\text{II}'\text{s} + 1\text{I}$ at Metaphase-I (0.4%)

Fig.12. $C_4 + 9\text{II}'\text{s}$ at Metaphase-I (0.5%) (X 1500)

Fig.13. Nondisjunction of chromosomes at late
Metaphase (0.4%) (X 1500)

PLATE — 35

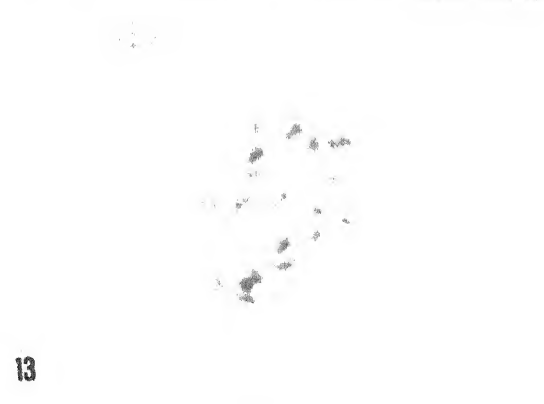
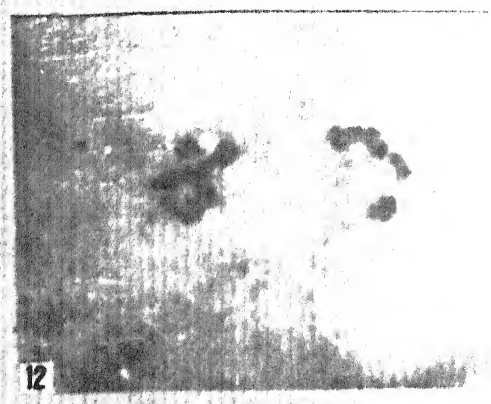
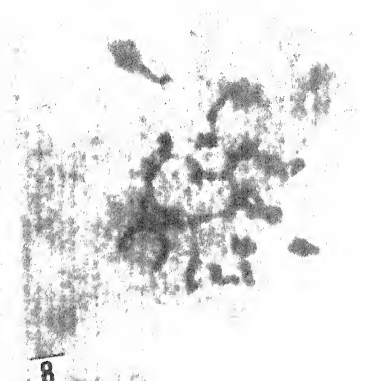
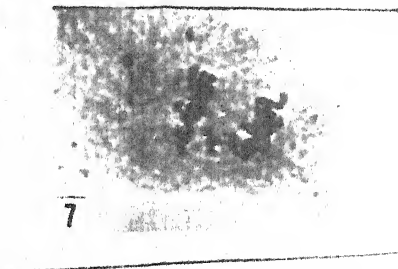
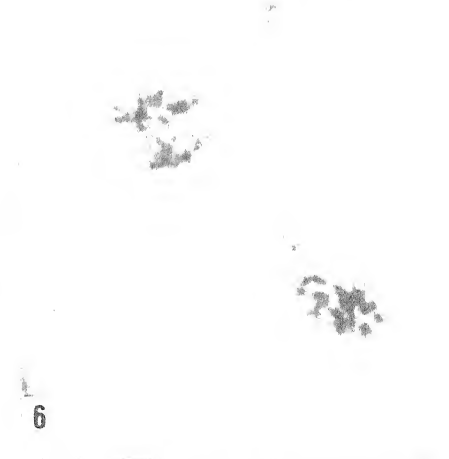
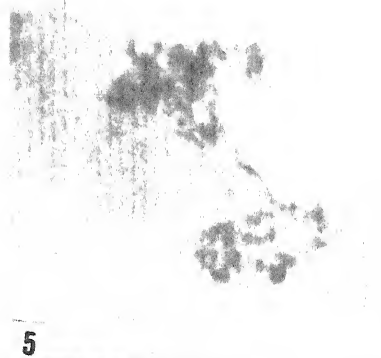
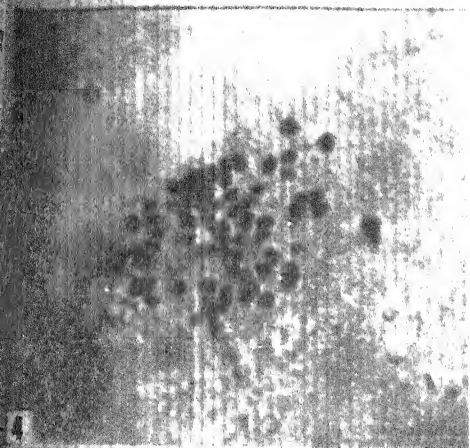
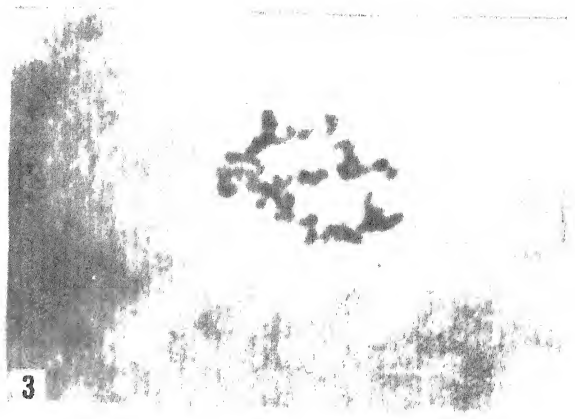
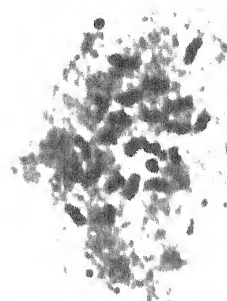
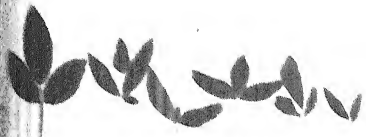


PLATE - 36 (Effect of EMS on C. cajan : ICP 8647 : Meiosis)

- Fig. 14. PMC's showing sticky groups of chromosomes at Metaphase-I (0.4%) (X 1500)
- Fig. 15. Unequal distribution of chromosomes at Anaphase-I (18-4) (0.6%) (X 1500)
- Fig. 16. 3 chromosomes away from the groups at Anaphase-I (0.6%) (X 1500)
- Fig. 17. Four unequal groups of chromatids at late Anaphase-II (X 1500)
- Fig. 18. Lagging fragments of chromosomes at Anaphase-II (0.6%) (X 1500)
- Fig. 19. Equal separation of chromatids in 4 groups at anaphase (0.4%) (X 1500)
- Fig. 20. Formation of 3 unequal groups at telophase-II with lagging fragments (0.6%) (X 1500)
- Fig. 21. Hexad (0.6%) (X 600)
- Fig. 22. Micronuclei with tetrads (X 600)
- Fig. 23. Pollen grains showing few sterile pollen grains (0.6%) (X 600)
- Fig. 24. Pollen grains showing partial sterility (0.6%) (X 600)

PLATE - 36



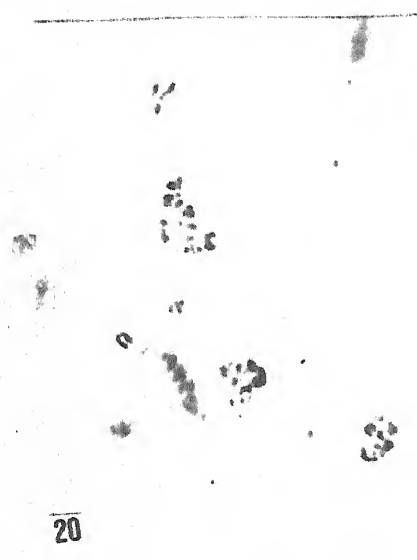
14



16



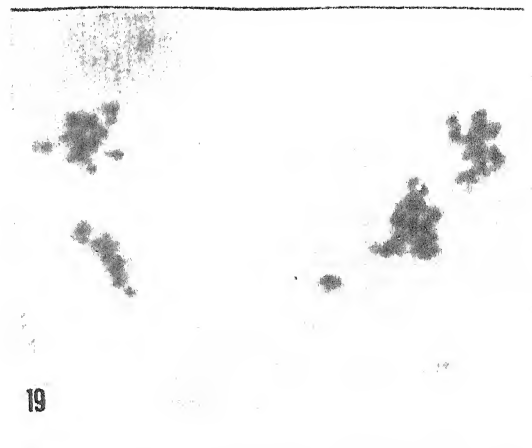
17



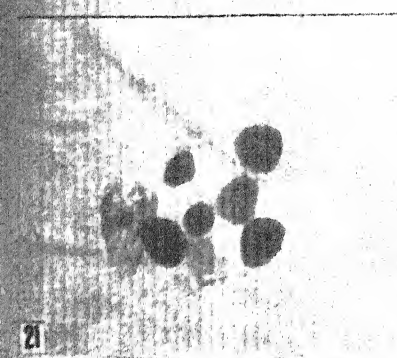
20



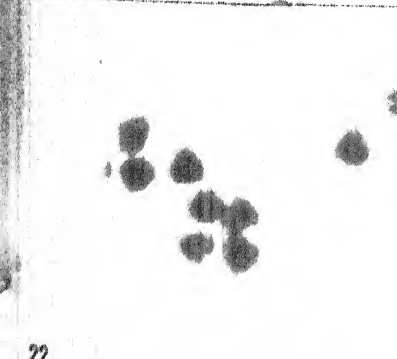
18



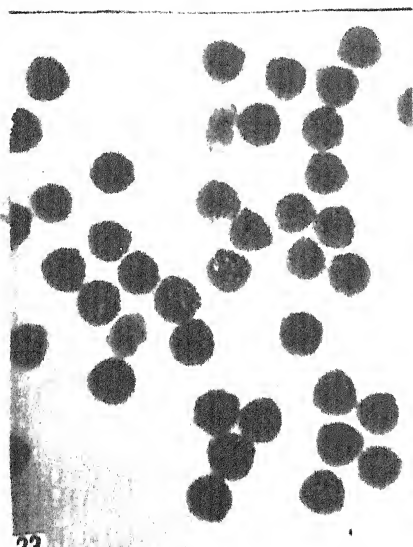
19



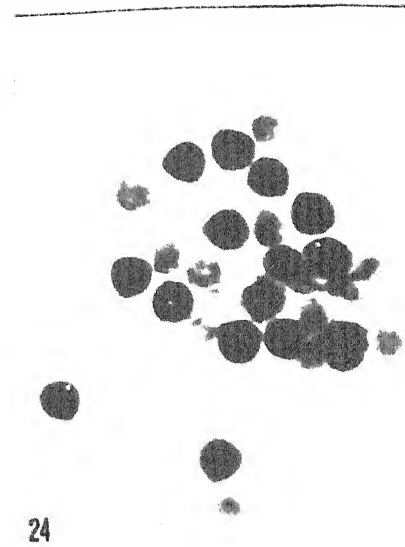
21



22



23



24

0.6 %:

Higher chromosome associations (hexavalent) were also noticed with the frequency of 0.02 per cell. At metaphase-I, quadrivalents and trivalents ranged from 0-1 and 0-1 with 0.05 and 0.02 per cell respectively. Increase in the frequency of rod bivalents and decrease in ring bivalents was the observable effects (Table-178). Univalents ranged from 0-2 with 0.05 per cell. Laggards at anaphase-I and II (Plate-36; Fig. 18) were recorded in 12.0 and 6.0 per cent cells respectively. At telophase-II, formation of 4 unequal daughter nuclei (Plate-36; Fig. 17) were recorded in 6.0% of cells.

Effect of EMS on seed germination and plant survival in *Calanus calan* (SMT coll.)

Observations on seed germination in petridished, emergence of plumules in field and survival to maturity in 4 and 8 hours treatments at different concentrations of EMS are as follows:

4 hours treatment:

After treatment with the lowest concentration (0.2%) of EMS solution, in comparison to control, a slight decline in the percentage of seed germination, plumule emergence and plant survival to maturity was recorded. At higher concentration (0.4%) further decrease in seed germination, plumule emergence and plant survival was registered (Table-179). At 0.6% concentration of EMS per cent seed germination, plumule emergence and plant survival were 45.0, 66.6 and 83.3 respectively. After the treatment with 0.8% EMS solution only 17.0 per cent seeds showed germination, while plumules could not emerge after

such a treatment. At the highest concentration of EMS solution (1.0%) no seed germination was recorded.

8 hours treatment:

The increased duration of treatment (8 hours) with lowest concentration (0.2%), has slightly reduced per cent seed germination, plumule emergence and plant survival till maturity. EMS at higher concentration (0.4%) reduced, seed germination, plumule emergence and plant survival till maturity as 75.0, 89.3 and 86.56 per cent respectively. At 0.6% concentration further decrease in such percentages was noticed (Table-179). At 0.8% concentration, only 15.0 per cent seeds germinated. No plumule could emerge after such a treatment. At the highest concentration of EMS (1.0%) no seed could germinate.

Reduction in the percentage of seed germination, plumule emergence and plant survival was linear with increase in concentration.

Morphological observations in EMS treated plants of *Cajanus cajan*.

Morphological observations in control, M_1 and M_2 plants of *Cajanus cajan* are summarised in Table-180. The details are as follows:

a) 4 hours treatment:

0.2 % :

M_1 plants had average 124 cm height, 5.1 primary and 16.0 secondary branches. Average central leaflet length was 4.5 cm and breadth 2.0 cm. Days to 50% flowering and

such a treatment. At the highest concentration of EMS solution (1.0%) no seed germination was recorded.

8 hours treatment:

The increased duration of treatment (8 hours) with lowest concentration (0.2%), has slightly reduced per cent seed germination, plumule emergence and plant survival till maturity. EMS at higher concentration (0.4%) reduced, seed germination, plumule emergence and plant survival till maturity as 75.0, 89.3 and 86.56 per cent respectively. At 0.6% concentration further decrease in such percentages was noticed (Table-179). At 0.8% concentration, only 15.0 per cent seeds germinated. No plumule could emerge after such a treatment. At the highest concentration of EMS (1.0%) no seed could germinate.

Reduction in the percentage of seed germination, plumule emergence and plant survival was linear with increase in concentration.

Morphological observations in EMS treated plants of *Cajanus cajan*.

Morphological observations in control, M_1 and M_2 plants of *Cajanus cajan* are summarised in Table-180. The details are as follows:

a) 4 hours treatment:

0.2 % :

M_1 plants had average 124 cm height, 3.1 primary and 16.0 secondary branches. Average central leaflet length was 4.5 cm and breadth 2.0 cm. Days to 50% flowering and

maturity were 107 and 182 respectively. Pods per plant was 30.2 and seeds per pod 3.1.

M_2 plants showed average 123.0 cm height, 6.2 primary branches and 17.8 secondary branches. Length of central leaflet was 4.6 cm and breadth 2.1 cm. Days to 50% flowering and maturity were nearer to those of control plants. Pods per plant was 36.1 and seeds per pod 3.2.

0.4 %:

M_1 plants showed 120.0 cm average height, 5.2 primary and 15.9 secondary branches. Length of central leaflet was 4.3 cm and breadth 2.0 cm. Days to 50% flowering and maturity were 10.7 and 18.8 respectively. Average number of pods per plant was 35.1 and seeds per pod 2.5.

M_2 plants had average 122.0 cm height, 7.4 primary and 16.2 secondary branches. On an average length of central leaflet was 4.5 cm and breadth 2.1 cm. Days to 50% flowering and maturity were 111 and 191 as against 105 and 183 in control plants. Pods per plant was 35.1 and seeds per pod 2.8.

0.6 %:

M_1 plants had 116.0 cm height, 3.0 primary and 12.8 secondary branches. Length of central leaflet was 4.2 cm and breadth 2.0 cm. Days to 50% flowering and maturity were 111 and 191 as against 105 and 183 in control plants. Pods per plant was 16.1 and seeds per pod 2.1.

M_2 plants showed 120 cm average height, 6.5 primary and 15.8 secondary branches. Length of central leaflet was

4.4 cm and breadth 2.0 cm. Days to 50% flowering and maturity were nearer to those of control plants. On an average number of pods per plant was 35.6 and seeds per pod 2.5.

8 hours treatment:

0.2 %:

M_1 plants showed 123.0 cm average height, 6.0 primary and 14.1 secondary branches. Length of central leaflet was 4.4 cm and breadth 1.9 cm. Days to 50% flowering were nearer to those of control plants. Pods per plant was 32.1 and seeds per pod 3.0.

M_2 plants had 124.0 cm average height, 7.1 primary and 18.2 secondary branches. Length of central leaflet was 4.5 cm and breadth 2.0 cm. On an average, number of pods per plant was 34.1 and seeds per pod 3.1.

0.4 %:

Average height of M_1 plant was 119.0 cm, primary branches 4.9 and secondary branches 13.0. Length of central leaflet was 4.2 cm and breadth 1.8 cm. Days to 50% flowering and maturity were 109 to 190. On an average number of pods per plant was 28.5 and seeds per pod 2.6.

M_2 plants had average height, 6.8 primary and 16.3 secondary branches. Length of central leaflet was 4.3 cm and breadth 2.0 cm. Days to 50% flowering and maturity was nearer to those of control plants. Pods per plant was 37.2 and seeds per pod 2.7.

0.6 % :

M_1 plants showed 115.0 cm average height, 3.8 primary and 10.0 secondary branches. Length of central leaflet was 9.0 cm and breadth 2.0 cm. Days to 50% flowering and maturity were 112 and 192 as against 105 and 183 in control plants. Pods per plant was 20.1 and seeds per pod 2.0.

M_2 plants had 117.0 cm average height, 6.5 primary and 16.1 secondary branches. Days to 50% flowering and maturity were nearer to those of control plants. Pods per plant was 30.2 and seeds per pod 2.5.

Thus in M_1 plants reduction in plant height, number of primary and secondary branches, pods per plant and seeds per pod was linear with increasing concentration and duration of EMS treatment (Table-180).

Cytology (M_1):Mitosis:

Chromosomal abnormalities detectable during mitotic divisions (Plate-37) are summarised in Table-181). Their details are as follows:

4 hours treatment:

At 0.2% concentration of EMS solution, chromosome stickiness (Plate-37; Fig. 2) and clumping at metaphase was observed in 2.0 and 4.0 per cent cells respectively. During anaphase no abnormality was recorded. At the next higher concentration (0.4%) chromosome stickiness, clumping and breakage (Plate-37; Fig. 3) was observed in 6.0, 4.0 and 4.0 per cent respectively. The treatment with 0.6%

concentration, further increased the percentage of cells showing chromosome stickiness, clumping and breakage (Table-181). In the treatment with the highest concentration (0.8%) cells upto 20.0 per cent were scored showing chromosome breakage. At anaphase, bridge with and without fragments were observed in 4.0 and 8.0 per cent cells respectively. Mitotic abnormality increased with the increasing dose of the chemical.

8 hours treatment:

After the treatment with 0.2% concentration almost normal mitosis was observed except in 2.0 per cent cells, where clumping and stickiness of chromosomes were scored. Treatment with 0.4% EMS solution revealed chromosome stickiness, clumping and breakage in 8.0, 6.0 and 4.0 per cent cells respectively. At anaphase bridge with fragments was observed in 4.0 per cent cells. At 0.6% concentration chromosome breakage was observed in 16.0 per cent cells. At anaphase, bridge with fragment and without fragment were observed in 4.0 and 6.0 per cent cells respectively. The highest concentration (0.8%) resulted in chromosome stickiness, clumping and breakage (Plate-37; Fig. 4) in 20.0, 12.0 and 24.0 per cent cells respectively. At anaphase increase in the percentage of cells showing bridge (Fig. 6, 7; Plate-37) with and without fragments was noticed (Table-181).

Thus a corresponding increase in chromosomal changes was observed at metaphase, and bridge with fragments at anaphase, with the increase in pods/duration of EMS treatment.

Meiosis (M₁ plants)

Observations on chromosomal configurations (Plate-37, 38) at each concentration and duration of treatment (Table-182) are as follows:

4 hours treatment:0.2 %:

No meiotic abnormality was recorded. However, very rarely univalents were formed. Pollen fertility was 93.72%.

0.4 %:

After this treatment, the multivalent frequency at M-I viz., quadrivalent was 0.16 and of trivalent 0.07. Ring and rod bivalents ranged from 5-11 and 0-6 with 3.61 and 6.83 per cell respectively. Univalents ranged from 0-3 with 0.21 per cell. At anaphase-I and II laggards were noticed in 4.77 and 1.65 per cent cells respectively. At sporad stage dyad, triad, tetrad and micronuclei were noticed. Pollen fertility was 88.61 per cent.

0.6 %:

Higher association of chromosomes viz., hexavalent (Plate-37; Fig. 8) also appeared in addition to quadrivalents and trivalent at M-I. Ring and rod bivalents ranged from 0-11 and 0-11 with 2.57 and 7.63 per cell respectively. Univalents ranged from 0-2 with 0.04 per cell. At anaphase-I and II laggards were observed in 10.84 and 3.21 per cent cells respectively. At sporad stage, dyad, triad, tetrad, polyad and micronuclei were noticed. Pollen fertility was 71.92 per cent.

8 hours treatment:0.2 %:

Meiosis was nearly normal except rare occurrence of two univalents at metaphase-I (0.22 per cell) and laggards at anaphase-I in 2.0 per cent cells. Pollen fertility was 91.51 per cent.

0.4%:

Meiotic abnormalities increased with increase in the concentration of the chemical. At metaphase-I frequency of quadrivalent was 0.05 per cell and of trivalent 0.02. Ring and rod bivalents ranged from 5-11 and 0-6 with 3.72 and 6.92 per cell respectively. Univalents at metaphase-I ranged from 0-3 with 0.08 per cell. At anaphase-I and II, laggards were observed in 5.00 and 4.0 per cent cells respectively. At sporad stage other than tetrads, dyad and triads were also noticed. Pollen fertility was 82.50 per cent.

0.6 %:

At metaphase-I hexavalent ranged from 0-1 with 0-2 per cell and quadrivalents (Plate-37; Fig. 11, 12) ranged from 0-4 with 0.12 per cell. Frequency of trivalents (Plate-37; Fig. 9) was 0.02 per cell. Increase in the range and frequency of rod bivalents was noticed (Table-182). Univalents ranged from 0-2 with 0.02 per cell. At metaphase-I most of the cells met with clumped fragments. In some cells unoriented chromatin mass was observed (Plate-38; Fig. 13). At this concentration some cells showed 3-4 times increased cell volume as compared to untreated ones (Plate-38; Fig. 17). At anaphase-I and II laggards (Plate-38; Fig. 15, 16) were recorded in 12.0 and 8.0 per cent cells respectively. At sporad stage dyad, triad, tetrad, polyad (Plate-38; Fig. 18, 19) and micronuclei were seen. Pollen fertility was 65.11 per cent. Meiotic anomalies progressively increased with the increase in concentration/duration of the treatment.

Table - 181

Mitotic observations in M_1 seeds of Cajanus cajan (SNT coll.)

| Concentration (%) | Duration (hrs.) | METAPHASE | | | | ANAPHASE | | | |
|-------------------|-----------------|----------------------|--|-------------|-------------|----------------------|-------------------|-------------|-------------------|
| | | No. of cells studied | Unaffected cells (2n = 22) breakage zone | Stickiness | Clumping | No. of cells studied | Normal separation | Bridge | Bridge + fragment |
| Control | - | 25 | 25 (100) | - | - | 25 | 25 (100) | - | - |
| 0.2 | 4 | 50 | 47 (94.0) | 1 (2.0) | 2 (4.0) | 30 | 30 (100) | - | - |
| 0.2 | 8 | 50 | 46 (92.0) | 1 (2.0) | 1 (2.0) | 30 | 30 (100) | - | - |
| 0.4 | 4 | 50 | 43 (86.0) | 2 (4.0) | 3 (6.0) | 29 | 29 (96.66) | 1 (3.33) | 1 (4.0) |
| 0.4 | 8 | 50 | 41 (82.0) | 2 (4.0) | 4 (8.0) | 23 | 23 (92.0) | 1 (4.00) | 2 (4.0) |
| 0.6 | 4 | 50 | 35 (70.0) | 7 (14.0) | 6 (12.0) | 50 | 45 (90.0) | 5 (10.0) | 1 (2.0) |
| 0.6 | 8 | 50 | 33 (66.0) | 8 (16.0) | 7 (14.0) | 50 | 45 (90.0) | 4 (8.0) | 1 (2.0) |
| 0.8 | 4 | 25 | 14 (56.0) | 5 (20.0) | 4 (16.0) | 25 | 23 (92.0) | 2 (8.0) | 2 (8.0) |
| 0.8 | 8 | 25 | 11 (44.0) | 6 (24.0) | 5 (20.0) | 25 | 20 (80.0) | 3 (12.0) | 2 (8.0) |

(Figures in parentheses are per cent)

Table - 182
 Meiotic observation in M_1 plants of Cajanus cajan (SNR coll.) No. of plants studied in each case were 5.

| Treat-ment (%) | Dura-tion of treat. (hrs.) | No. of cells studied | Chromosomal associations at | | | | | | Anaphase I | | Anaphase II | | Pollen fertility (%) |
|----------------|----------------------------|----------------------|-----------------------------|------------|------------|-------------|-------------|------------|----------------------|------------|----------------------|------------|----------------------|
| | | | Metaphase - I | | | | | | No. of cells studied | Laggs. (%) | No. of cells studied | Laggs. (%) | |
| | | | VI | IV | III | Ring II | Red II | I | | | | | |
| Control | - | 50 | - | - | - | 9-11 (10.5) | 0-2 (0.5) | - | 50 | - | 80 | - | 99.18 |
| 0.2 | 4 | 40 | - | - | - | 7-11 (8.40) | 0-4 (2.50) | 0-2 (0.20) | 85 | 1.11 | 70 | - | 93.72 |
| 0.2 | 8 | 45 | - | - | - | 7-11 (8.63) | 0-4 (2.25) | 0-2 (0.22) | 50 | 2.00 | 71 | - | 91.51 |
| 0.4 | 4 | 42 | - | 0-2 (0.16) | 0-1 (0.07) | 5-11 (3.61) | 0-6 (6.83) | 0-3 (0.21) | 125 | 4.77 | 63 | 1.65 | 88.51 |
| 0.4 | 8 | 34 | - | 0-2 (0.05) | 0-1 (0.02) | 5-11 (3.72) | 0-6 (6.92) | 0-3 (0.08) | 100 | 5.00 | 50 | 4.00 | 82.50 |
| 0.6 | 4 | 47 | 0-1 (0.06) | 0-4 (0.21) | 0-1 (0.04) | 0-11 (2.57) | 0-11 (7.63) | 0-2 (0.04) | 68 | 10.85 | 68 | 3.21 | 71.92 |
| 0.6 | 8 | 40 | 0-1 (0.02) | 0-4 (0.12) | 0-1 (0.02) | 0-11 (2.97) | 0-11 (7.85) | 0-2 (0.02) | 50 | 12.00 | 50 | 8.00 | 65.11 |

(Mean values in parentheses)

Table - 179

Germination of EMS treated seeds of Cajanus cajan
(SNT Coll.) (No. of seeds treated in each case was 50)

| Concen- tration of EMS (%) | Duration of treat- ment (hours) | Germination in petridish (%) | Emergence of plumule in field (%) | Survival to maturity (%) |
|-------------------------------------|--|------------------------------------|--|--------------------------------|
| Control | - | 94.0 | 97.87 | 97.82 |
| 0.2 | 4 | 88.0 | 90.90 | 95.0 |
| " | 8 | 86.0 | 88.37 | 90.78 |
| 0.4 | 4 | 76.0 | 90.78 | 86.95 |
| " | 8 | 75.0 | 89.33 | 86.56 |
| 0.6 | 4 | 45.0 | 66.66 | 83.33 |
| " | 8 | 43.0 | 65.11 | 71.42 |
| 0.8 | 4 | 17.0 | 11.76 | NIL |
| " | 8 | 15.0 | 13.33 | NIL |
| 1.0 | 4 | NIL | - | - |
| " | 8 | NIL | - | - |

Morphological observations in control, M₁ and M₂ plants of Calanthe calan (SWT Coll. -)

| Characters | Control | Gene- ration * | 4 hours treatment | | | 8 hours treatment | | |
|--------------------------------|---------|----------------------|-------------------|---------|---------|-------------------|---------|---------|
| | | | 0.2% | 0.4% | 0.6% | 0.2% | 0.4% | 0.6% |
| Plant height (cm) | 125 | M ₁ | 124 | 120 | 116 | 123 | 119 | 115 |
| | 122 | M ₂ | 123 | 122 | 120 | 124 | 120 | 117 |
| No. of primary branches | 6.2 | M ₁ | 5.1 | 5.2 | 3.0 | 6.0 | 4.9 | 3.8 |
| | 8.3 | M ₂ | 6.2 | 7.4 | 6.5 | 7.1 | 6.8 | 6.5 |
| No. of secondary branches | 16.6 | M ₁ | 16.0 | 15.9 | 12.8 | 14.1 | 13.0 | 10.0 |
| | 18.1 | M ₂ | 17.8 | 16.2 | 15.8 | 18.2 | 16.3 | 16.1 |
| Central leaflet (L x B) cm. | 4.6x2.0 | M ₁ | 4.5x2.0 | 4.3x2.0 | 4.2x2.0 | 4.4x1.9 | 4.2x1.8 | 4.0x2.0 |
| | 4.5x2.1 | M ₂ | 4.6x2.1 | 4.5x2.1 | 4.4x2.0 | 4.5x2.0 | 4.3x2.0 | 4.1x2.1 |
| Days to flowering | 105 | M ₁ | 107 | 107 | 111 | 104 | 109 | 112 |
| | 107 | M ₂ | 105 | 106 | 106 | 105 | 107 | 106 |
| Days to maturity | 183 | M ₁ | 182 | 188 | 191 | 183 | 190 | 192 |
| | 185 | M ₂ | 184 | 184 | 185 | 184 | 185 | 186 |
| Pods per plant | 33.5 | M ₁ | 30.2 | 25.2 | 16.1 | 32.1 | 28.5 | 20.1 |
| | 35.1 | M ₂ | 36.1 | 35.1 | 35.6 | 34.1 | 37.2 | 30.2 |
| Seeds per pod | 3.1 | M ₁ | 3.1 | 2.5 | 2.1 | 3.0 | 2.6 | 2.0 |
| | 3.2 | M ₂ | 3.2 | 2.8 | 2.5 | 3.1 | 2.7 | 2.5 |

* In each generation 10 plants were studied

PLATE - 37 (Effects of EMS on C. cajan (SNT Coll.))

Fig. 2-7 : Mitosis; 8 to 11 Meiosis.

Fig. 1. Morphological variation in leaflet number and shape.

Fig. 2. Chromosomes showing stickiness at Metaphase. (0.2%) (X 1500)

Fig. 3. Chromosome breakage at Metaphase-I (0.6%) (X 1500)

Fig. 4. Heavy chromosome fragmentation at Metaphase (0.8%) (X 1500)

Fig. 5. Non-disjunction of Chromosomes at late metaphase (0.4%) (X 1500)

Fig. 6. Paired chromatid bridge at Anaphase-I, without fragment (X 1500)

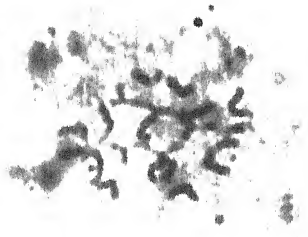
Fig. 7. Multiple bridges with fragments at Anaphase (X 1500)

Fig. 8. 1 IV + 8 II's at Metaphase-I (0.6%) (X 1500)

Fig. 9. 1 III + 9 II's + 1I at Metaphase-I (0.4%) (X 1500)

Fig. 10. 3 C₄ + 4 II's + 2 I's at Metaphase-I (0.6%) (X 1500)

Fig. 11. 1 IV + 9 II's at Metaphase-I (0.6%) (X 1500)



5



2



3

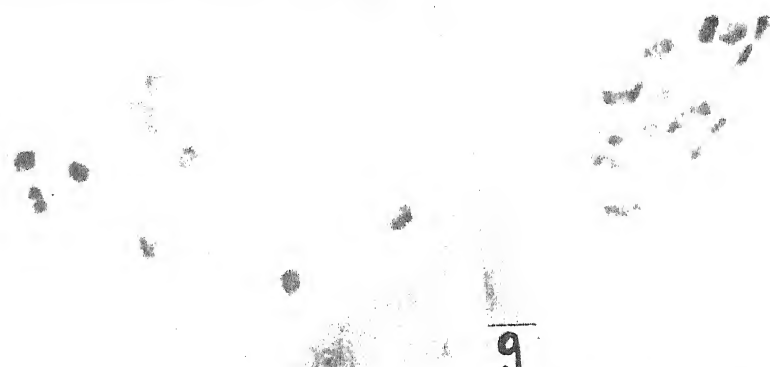


6



7

8



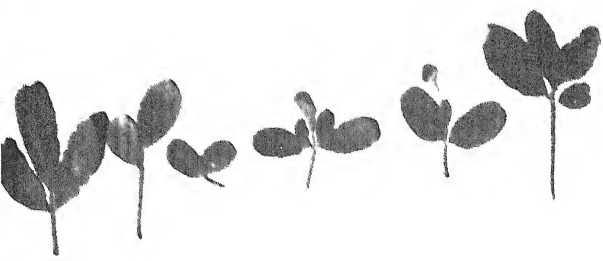
9



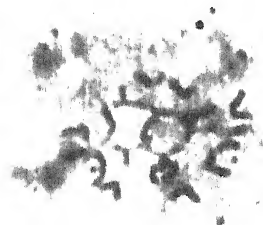
11

10

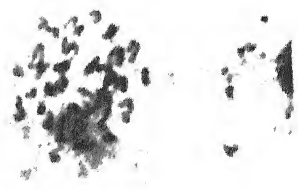
PLATE - 37



1



2



5



4



3

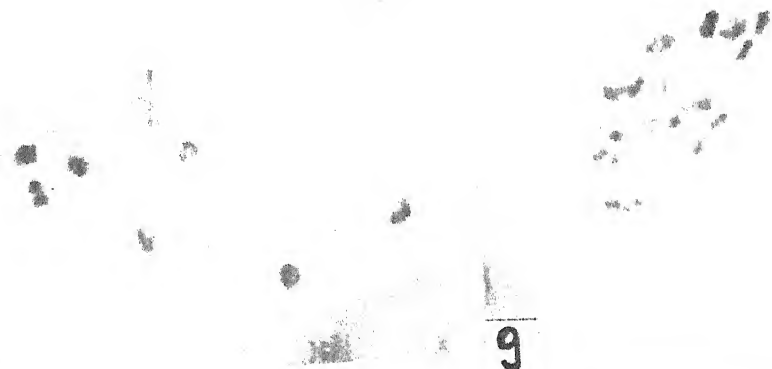


6

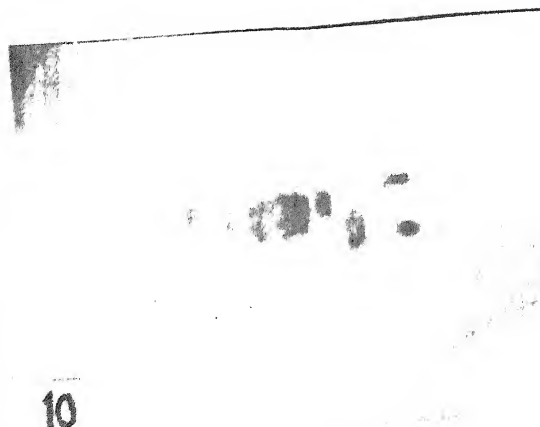


7

8



9



10



11

PLATE - 38 (Effect of DMS on P. sojan (SNT 7511.))

Fig. 12. PMC's showing chromosome fragmentation (0.6%) (x 1500)

Fig. 13. Unoriented mass of chromatin material (0.6%) (x 1500)

Fig. 14. Laggaris at Anaphase-I (0.6%) (x 1500)

Fig. 15. Lagging fragments at Anaphase-I (x 1500)

Fig. 16. Laggaris at Anaphase-II (x 1000)

Fig. 17. Giant pollen mother cell at Anaphase-II (x 70)

Fig. 18. Hexad and normal tetrad (x 600)

Fig. 19. Dyad, triad and tetrad (x 600)

Fig. 20. Stainable pollen grains showing variation in size (x 600)

Fig. 21. Pollen grains of untreated plant. (x 600)

PLATE — 38

T Coll.)

tation (0.0)

aterial

1500)

(X 1500)

ase-II

ariation in

(X 600)

12

13

14

15

16

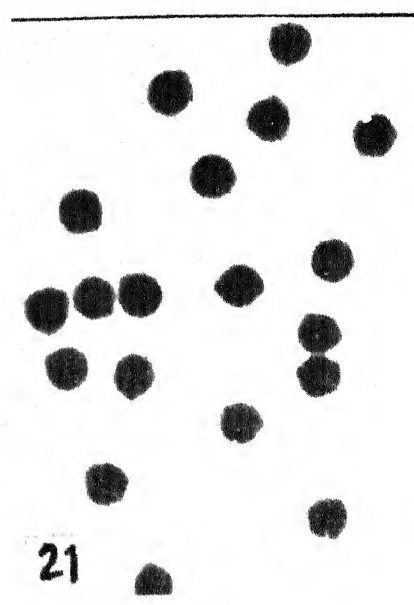
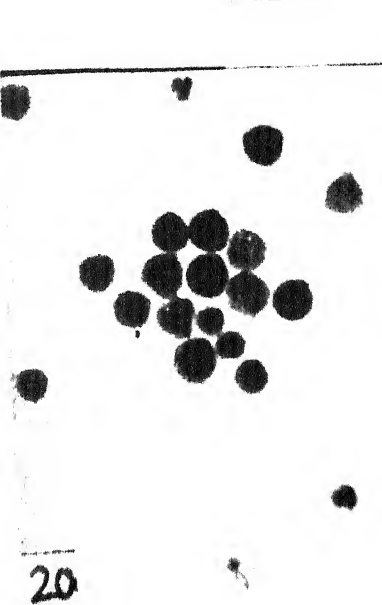
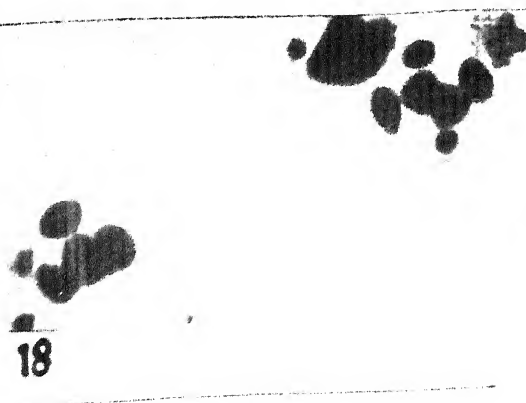
17

18

19

20

21



DISCUSSION

The genus Atylosia has largely been classified on external morphological characteristics (Hooker, 1975). Cajanus cajan Linn. (Millsp.) was mostly considered to be monotypic genus (Hooker, 1875) because Cajanus kerstingii Harms described in 1915 from West Africa was unknown to most agricultural scientists. At present only two species— the cultivated Cajanus cajan (L). Millsp and the wild Cajanus kerstingii Harms are classified by DeCondolle (1813). The genus Atylosia is closely related to Cajanus cajan (Wight and Arnott, 1834) and separated from the latter on the basis of the presence^{of} strophioled seeds (Baker, 1876). In fact the strophiole is not altogether absent in pigeonpea. The sole criterion for distinction between the two genera do not hold qualified as more than 144 accessions of pigeon pea maintained at ICRISAT do possess small strophiole on seeds (Vander Maesen, 1980).

Atylosia cajanifolia, Haines described in 1920 from the Puri forest in India resembles pigeon pea so much that casual observers might assume, it is pigeon pea escaped from cultivation (Vandermaesen, 1980). A. cajanifolia, on morphological basis seems to be the closest relative of pigeon pea. Several Atylosia species are cross compatible with pigeon pea. Such combining ability of Atylosia with Cajanus makes an important point of consideration for their congenricity.

Chromosome number and morphology:

Cytogenetical data provide valuable clues for assesment of phylogeny and evolutionary status of a genus

or species and the studies made by Babcock (1947) on Crepis, Navashin (1926) and Cleland (1962) on Oenothera, Mather (1932) on Crocus, Manton (1932) on Cruciferae, Levan (1931, 1932, 1934, 1935a, b) on Allium, Deodikar and Thakar (1956), Reddy (1973) on Cajanus and Atylosia, Pundir (1981) (1985 a, b, c) on Cajanus, Atylosia and Rhynchosia are some of the classical examples in this regard.

Navashin (1926) observed that most species of living organisms show a distinct and constant individuality of their somatic chromosomes and that closely related species have more similar chromosomes than those of distantly related ones. Darlington (1963) and Stebbins (1950, 1971) have discussed in detail on some aspects of chromosome morphology which help in understanding the evolutionary problems. According to Sharma (1985) changes in chromosome morphology shows alterations in gene arrangement which influence their subsequent segregation and recombination. Chromosomal variation thus reflects differences in the source or genic variation, while morphological differences indicate variation in the products of gene action as modified by environmental factors.

The karyotype was first defined in 1926 by Delaunay as group of species resembling each other in the morphology and number of their chromosomes. However, Levitsky (1924, 1931) opined that the evolution of Karyotype in many genera takes place through a series of alterations in chromosome morphology. According to him, karyotype is the phenotypic appearance of the somatic chromosomes, in contrast to their genotype.

In the present studies, ^{twenty} two somatic chromosomes were recorded in all the species of Atylosia, and Cajanus

Their karyotypic studies also revealed presence of almost similar chromosomes in both the genera i.e., Atylosia and Cajanus. Only minor differences were observed in the chromosomes of different species of Atylosia and Cajanus cajan.

The presence of identical karyotypes in different genera of Liliaceae has been reported by Delaunay (1926). Levitzky (1924, 1931) had contradicted the observations of Delaunay and suggested that minor differences did exist within a genus. The different species of Paeonia have all alike chromosomes, but the study of their hybrids indicated translocations and inversions in them (Stebbins, 1938). Many workers viz., Bose, 1957; Sharma, 1956; Sharma and Bhattacharjee, 1957; Sharma and Sharma, 1959; and Sharma, 1985 have substantially highlighted the role of chromosome alterations in evolution and differentiation of species and varieties. Cytological studies on several species of Phaseolus and Vigna by Joseph and Bouolemt (1978) and Frahm-Leliveld (1965) have revealed symmetrical karyotypes with little differences in shortest and longest chromosomes, as well as poor gradation in size.

The karyotypic analysis of the 13 varieties of Pigeon pea revealed considerable intervarietal variation in chromosome complement of the species in regard to arm ratio, total length and T.F. % (Srivastava et al., 1973). These workers have also highlighted there are no major differences in karyomorphology of a large group of Pigeon pea varieties and the closely related Atylosia lineata. In the present study it was noticed that there was no major difference in the karyotype of Atylosia species and Cajanus cajan. The wide variation in pigeon pea and Atylosia lineata might have generally resulted from gross changes in karyomorphology.

Sinha and Kumar 1979 in their study of mitotic analysis of 15 varieties of C. cajan reported chromosome

number $2n = 22$. In the present study, the total chromatin length of *Atylosia* species ranged from 40.86 to 81.60 μ and in cultivar/collection of Cajanus cajan from 53.16 to 59.12 μ . The differences in the total chromatin length can be considered as one of the most important factor in their evolutionary history. According to Babcock (1947) and Cameron (1934) decrease in total chromatin length is one of the factor responsible for evolution and hence A. lineata having minimum (40.86 μ) chromatin length may be considered as most advance/and A. albicans with maximum chromatin length (81.60 μ) as most primitive (Table-1). Such a reduction in chromatin length appears to occur due to the erosion of the chromatid segments during the process of evolution. The total chromatin length is a feature which is under genetic control (Sharma, 1978). Somatic studies made by Shrivastava et al. (1973) in different varieties of pigeon pea have shown variation in total chromatin length (27.6 to 44.9 μ) and Sinha and Kumar reported variation in total chromatin length from 35.4 to 51.2 μ in 15 varieties of Cajanus cajan.

It has been argued that progressive physiochemical diversification of a more or less constant chromosomal substances rather than increase in the number of gene loci contained on each chromosomes has perhaps been responsible for increasing evolutionary diversification (Stebbins, 1950). The differences in the absolute chromosome size are to some extent controlled by factors outside the chromosome themselves. Pierce (1937) found that lack of phosphorus in nutrition of plants causes considerable reduction in the size of chromosomes of Viola.

When the karyotypic assymetry is taken into account, the asymmetrical karyotypes are supposed to be more advanced

than symmetrical ones (Stebbins, 1950) and therefore, the species having maximum number of submedian chromosomes (assymetrical) is more advanced than that having minimum number of submedian chromosomes. In the present study, A. scarabaeoides having maximum (8) submedian chromosome pairs can be considered as most advanced species in comparison to other Atylosia species. Cajanus cajan (ICP 8647) appears to be more advanced showing 7 submedian chromosome pairs in comparison to the C. cajan (SNT colle.) which possess 6 pairs of submedian chromosomes.

On the basis of T.F. % also, cultivar (ICP 8647) of C. cajan tends to be most advanced having the lowest T.F. % (40.37). Atylosia scarabaeoides (R.J.W. coll.) possessing the largest T.F. % (43.40) may be considered as the most primitive one in comparison to other Atylosia spp., presently studied.

Sinha and Kumar (1979) in a study of 13 varieties of pigeon pea reported only one pair of satellited chromosome in 4 varieties. Similarly the present study on Cajanus cajan (SNT coll.) showed absence of satellited chromosomes. The absence of secondary constriction may be attributed to the fact that accidental hybridization and translocation might have occurred during the course of evolution resulting in the elimination of SAT chromosomes. Earlier absence of SAT chromosomes were reported by Delauney (1926) and Sinha and Acharia (1974) in Lens nigricans. Further more, studies made by Sharma and Gupta (1982) on karyotype of pigeon pea variety I-21 and Deodkar and Thakar (1956) in A. lineata, revealed no SAT chromosomes.

Chromosomes of Atylosia species and Cajanus cajan however, do not vary greatly in their centromeric position

as shown by Reddy (1978) in Atylosia lineata, A. scarabaeoides and A. sericea; Sikdar and De (1967) in A. scarabaeoides and A. lineata. In the present study, all the Atylosia species and Cajanus cajan showed 2-4 median, 6-8 submedian and 1-2 subterminal chromosome pairs. Presence of subterminal centromeres could probably be due to deletion and deficiency in one arm of the chromosomes causing the shift in centromeric position. This shifting of centromeric position may not be in a similar fashion in all the chromosomes of the same species/cultivar and thus lead to assymetric condition of the karyotypes. This karyotypic evolution brought about by repatterning of chromosomes might be considered as one of the prime factors for evolution within the same species and thus the formation of different varieties with the same chromosome number.

Sinha and Kumar (1979) in their studies on 6 varieties of Cajanus cajan reported $2n = 22$ in all the 6 varieties but these varieties differed in their chromosome morphology and also total chromatin length. Variation in morphological characters observed among the cultivars of pigeon pea sometimes associated with variation in the karyotypes. For instance, Srivastava et al. (1973) observed that the tallest of the fifteen varieties of C. cajan possessed the highest value of total chromatin length. In the present study, A. albicans showed maximum total chromatin length with maximum plant spread in comparison to other Atylosia species. According to Srivastava et al (1973) absence of satellite in variety P-958 of Cajanus cajan was associated with trifoliate leaves and in the present observation oval-oblong leaves of Cajanus cajan (SNT colle.) was associated with absence of satellite. However, such an association between morphological traits and features of karyotypes was not

always available. No. large difference in chromosome assymetry exist between annual and perennial species. Similar results on the differences in karyotypes of different varieties have been reported by Ghosh (1964) in *Oryza* by Sen and Tiwari (1966) in *Pisum sativum* and by Jagthe San and Ratnumbal (1969) in *Saccharum robustum*.

Similarities in karyotypes of *Cajanus cajan* with those of *A. lineata* have been observed by several workers (Deodikar and Thakar, 1956; Sikdar and De, 1967; Kumar et al 1958). The similarities included the presence of satellite in the longest chromosome in both the genus i.e. *Atylosia* and *Cajanus*.

Pundir (1981) reported two pairs of satellited chromosomes in *A. albicans* and *A. lineata*. In the present study, two pairs of satellited chromosomes were noticed in *A. lineata* (JM 3366), *A. volubilis* (JM 1984) and *Cajanus cajan* (ICP 8647). Two pairs of SAT chromosomes were also reported in *Cajanus cajan* by Upadhyay (1986) and Pundir (1985). Present study revealed one pair of SAT chromosomes in *A. lineata* (JM 2639), *A. scarabaeoides*, *A. platycarpa*, *A. albicans*, *A. mollis* and *A. cajanifolia*.

According to Stebbins (1971) increased specialization in many plant genera is associated with decreasing karyotype symmetry. From this point of view the present investigation shows that *Cajanus cajan* is advanced and *Atylosia* species are primitive or represents a trend towards increasing chromosome symmetry.

Darlington (1939) has pointed out that the amount of genetic recombinations in any particular intermating group is determined by the chromosome number of the species

and by the amount of crossing over in each chromosomes which in turn, is determined by chiasma frequency. The somatic chromosome number in pigeon pea is confirmed to be $2n = 22$ with a base number of $n = 11$ (Roy, 1933; krishnaswamy and Ayyangar, 1935; Naithani, 1981; Kumar *et al.*, 1958; Shrivastava *et al.*, 1972; Sinha and Kumar, 1971, 1979b and Akinola, 1973. Chromosome number ($2n = 2x = 22$) in Atylosia species has been reported by Reddy (1981 a, b, c); Kumar *et al.*, (1984) Tripathi *et al.*, 1984., Pundir (1985), Dundas (1985), Mukhopadhyay (1986), Jha (1986), and Tripathi, 1986.

Results of the present studies also confirm the same chromosome number in Calanus and seven species of Atylosia. Darlington (1965) has pointed out that chiasma frequency is directly proportional to chromosome length. In the present studies 1.6 to 1.9 chiasma per bivalent in Atylosia species and Calanus calan were recorded. Which is in accordance with the report in Calanus calan by Upadhyay (1985) and Atylosia scarabaeoides by Jha (1986).

In the present study meiotic stages followed normal course of cell division at diakinesis, metaphase-I, anaphase-I and II, telophase-II spored stage and pollen formation in all the species of Atylosia and Calanus. Normalization of meiotic phases and pollen fertility may be dependent upon the level of adaptation of a member to environmental factors. Equal separation of chromosomes and equal cytokinesis resulted in very less variation in the size of pollen grains and resulted in high pollen and ovule fertility.

Table - I

Karyotypic analysis of different species of Atylosia and Cajanus cajan

| species | Somatic chromosome number | Range of chromosome length in (μ) | Total chromatin length in (μ) | No. of chromosome pairs | | | T.F (per cent) | L/S ratio |
|--|---------------------------|---|-------------------------------------|-------------------------|----|----|----------------|-----------|
| | | | | M | SM | ST | | |
| <u>Cajanus cajan</u> (SNT coll.) | 22 | 1.41-3.54 | 53.16 | 3 | 6 | 2 | 42.04 | 1.00-2.00 |
| <u>C. cajan</u> (ICP 8647) | 22 | 1.77-4.25 | 67.36 | 2 | 7 | 2 | 43.14 | 1.00-2.33 |
| <u>A. lineata</u> (JM 2639) | 22 | 1.05-2.13 | 40.86 | 2 | 7 | 2 | 43.31 | 1.00-2.02 |
| <u>A. lineata</u> (JM 3366) | 22 | 1.42-2.84 | 49.26 | 4 | 5 | 2 | 43.07 | 0.71-3.00 |
| <u>A. platycarpa</u> (JM 2873) | 22 | 1.70-3.55 | 51.14 | 3 | 6 | 2 | 40.43 | 1.00-3.00 |
| <u>A. scarabaeoides</u> (RJW coll.) | 22 | 1.91-3.53 | 56.40 | 2 | 8 | 1 | 43.40 | 1.00-2.00 |
| <u>A. mollis</u> (JM 2943) | 22 | 1.77-3.54 | 56.76 | 3 | 6 | 2 | 42.98 | 1.00-3.00 |
| <u>A. volubilis</u> (JM1984) | 22 | 2.13-3.90 | 63.14 | 2 | 7 | 2 | 40.63 | 1.00-3.00 |
| <u>A. cajanifolia</u> (JM 2739) | 22 | 2.48-3.54 | 63.60 | 3 | 6 | 2 | 42.78 | 1.00-3.00 |
| <u>A. albicans</u> (JM 2337) | 22 | 2.13-3.56 | 81.60 | 2 | 7 | 2 | 40.78 | 1.00-2.02 |

Crossability:

It is well known that the crossability is a pre-requisite for gene transfer. However, an understanding on the extent of barriers to crossability among the species has been helpful in choosing methods for producing hybrids and their derivatives and also in tracing phylogenetic relations.

Crossing techniques have been discussed by Wilson, 1972; and Solh et al., 1980. Solh et al., (1980) have studied the effects of timing of emasculation and pollination on success of the crosses in lentil. They have adopted the following 4 time schedule in their experiments.

- T₁ : Morning emasculation and immediate pollination (before 10.0 a.m.)
- T₂ : After noon emasculation and immediate pollination (1.0 p.m. to 4.0 p.m.)
- T₃ : Morning emasculation and evening pollination
- T₄ : Afternoon emasculation and morning pollination.

The experimental results of Solh et al., (1980) revealed that T₁ type of crosses were successful. Similarly in the present study on the members of the subtribe cajaninae maximum success was achieved in the crossing schedule following morning emasculation and immediate pollination. The success in crossing by pollination just after emasculation has also been reported by Pundir and Singh (1985) in the study of crossability relationship among Cajanus, Atylosia and Rhynchosia and Tripathi et al., (1984) while making crosses between Atylosia cajanifolia and Cajanus cajan Veeraswamy and Sherief (1973) also reported that optimum hours for successful hybridization

in red gram are between 10.00 a.m. to 12.00 noon.

The studies on species hybrids have proved to be extremely useful in understanding the interrelationship between the species. The new systematic recognizes the morphological, physiological, ecological or ethological differences in genetic constitution (Magoon *et al.*, 1962). Further, the essence of speciation is accepted to lie in the development of barriers which present or restrict to a great extent, the free exchange of genes between two mandelian populations and such reproductive isolation is considered essential before two groups can be referred to different species (Mayer, 1948).

The present study on interspecific hybridization was undertaken and the cross compatibility of species, their interrelationship, based on cytomorphological characters were examined. The degree of crossability between Atylosia species as judged by percentage success of each cross was the highest in the cross between A. platycarpa and A. mollis, followed by A. lineata and A. cajanifolia, A. albicans and A. cajanifolia and A. lineata and A. albicans (Table-II). The success in interspecific crosses between Atylosia species has earlier been reported by Tripathi and Patil (1984) and Pundir and Singh (1985). In the present study, interspecific crosses made between Atylosia species, revealed unidirectional success. Similarly, unidirectional success in interspecific crosses is also reported in Phaseolus by many workers (Strand, 1943; Lorz (1952); Honma, (1958); Sen and Ghosh (1960); Baishand (1956); Dana (1964) and 1965) Likewise, in Nicotiana by Kostoff (1943); Swaminathan and Murthy (1957), and in Arachis by Muhammed (1970).

Out of 29 crosses attempted between Atylosia species, percentage success of crossability ranged from 0.26 (A. lineata x A. albicans) to 6.0 (A. platycarpa x A. mollis). The earlier reports on similar aspects by Pundir and Singh (1985) on percentage success of crossability in A. scarabaeoides x A. serices and A. lineata x A. albicans ranged from 0.6 to 12.0

The genus Atylosia is closely related to Cajanus cajan. The close affinity between Atylosia and Cajanus has been substantiated by their successful hybridization (Deodikar and Thakar, 1956; Kumar and Thombre, 1958; Kumar *et al.*, 1958; 1966; Sikdar and De, 1967; Reddy, 1973; De, 1974; Ariyanayagam and Spence, 1978; Reddy *et al.*, 1981; Tripathi *et al.*, 1984; 1986; Pundir and Singh, 1985; Kumar *et al.*, 1985; Dundas, 1985; Yadav *et al.*, 1986). Some of the Atylosia species possesses very valuable characteristics that are lacking in pigeon pea cultivars, such as A. scarabaeoides (L.) Bth. possess both physical and antibiosis type of resistance to the pod borer, Heliothis armigera, A. albicans ♂ & A. are rich in protein (Reddy *et al.*, 1979), A. lineata, wilt resistant (Remanandan, 1980), A. volubilis (Blanco) gamble, sterility mosaic resistant and high seed protein content (Remanandan, 1980) A. platycarpa (Benth) blight resistance (Remanandan, 1980).

In the present studies, in all the 25 interspecific failed cross combinations, flowers shed on the ground after 3 to 7 days of pollination. It may be due to the formation of abscission layer at the base of the pedicel of flower and pod. In the crosses A. albicans (♀) and A. volubilis (♂), pollen germinated on the stigmas but pollen tube growth was inhibited inside the style or stigma. In other unsuccessful interspecific cross combinations pollen germinated on the stigma but pollen tube growth inhibits

on stigmatic surface suggest that interspecific incompatibility exist in the material studied.

The present study on intergeneric hybridization was under taken and crossability of Atylosia species with Cajanus cajan was studied. In 3 successful intergeneric crosses of the 12 combinations attempted the crossability was 0.6 per cent in (A. scarabaeoides x C. cajan) and 2.8 per cent in (A. lineata x C. cajan). The degree of crossability as judged by per cent success of crossability of each cross was highest in case of A. lineata x C. cajan followed by A. albicans x C. cajan and A. scarabaeoides x C. cajan (Table-III). Crosses were attempted in both the directions but success were unidirectional (Atylosia species being female parent). Successful intergeneric crosses using Atylosia species as female parent and C. cajan as male parent were earlier reported by many workers (Ariyanayagam and Spence, 1978; Tripathi, 1984; Pundir, 1985a).

In the present study, no success was obtained using C. cajan as a female parent. The failure in intergeneric crosses, using C. cajan as a female parent can probably be attributed to the fact that the gene mutations and selection pressures under domestication underlying the evolution of the cultivated taxon have probably resulted in the accumulation of modifiers and differentiation of plasmon in the cultivated taxon (C. cajan). These changes render C. cajan as an unsuccessful seed parent when cross pollinated with Atylosia species. However, Kumar et al. (1985), Kumar and Thombre (1958), Roy (1966), Reddy (1980), Kumar et al., (1985), Yadav (1986), obtained success using C. cajan as a seed parent, when cross pollinated with Atylosia species. This discrepancy may be due to different genotypes used in the crosses.

In the crosses of C. cajanus cajan with Atylosia platycarpa, A. mollis and A. volubilis seedless pods were obtained. Cross failure may exist at the gametic, Zygotic or post zygotic levels including hybrid sterility and weakness (Pundir, 1985). In the present study it has been observed that hybrid inviability in the crosses of A. platycarpa, A. volubilis and A. mollis with Cajanus cajan was an active barrier at post fertilization stages. Hybrid inviability originates usually from physiological incompatibility between embryo, endosperm and maternal tissue. This reaction at early stages leads to abortion of young hybrid embryo in case of A. mollis x C. cajan and at later stages results in the formation partially filled hybrid univiable seeds. Both actions results in the production of non-viable empty seeds and partly viable partially filled seeds in the present crosses. Similar condition i.e. pods having partially filled seeds was also reported by Dane (1966) in the studies of the cross between Phaseolus species, and Kumar et al., (1985) found extremely shrivelled seeds, in the hybridization of Atylosia sop x C. cajan, which did not germinate.

Morphology of hybrids:

The F_1 hybrid plant exhibited dominant recessive relationship for some qualitative characters, vigour for some quantitative characters and intermediate expressions for others. The F_1 hybrid plant of A. albicans x A. lineata was semi-erect/spreading in growth habit. Characters of A. lineata viz., presence of purple stripes on the yellow standard petal, hairy surface of pod, brown with black dotted seed coat colour were dominant to those of A. albicans. Characters like leaf shape, leaf size, petiole length were intermediate in F_1 hybrid of A. lineata (♀) x A. albicans (♂).

In F_2 generation, flower colour segregated in 3:1 (3 yellow with red stripes : 1 yellow) ratio. In the cross between A. lineata (σ) x A. cajanifolia (σ^7) characters of A. cajanifolia viz., red colour of standard petal, lanceolate shape of first pair of leaves, dark brown colour of pod and red colour of seeds were dominant over to those of A. lineata. This F_1 hybrid showed vigour for plant height, leaf length and breadth, number of primary and secondary branches and flower size. In F_2 generation; segregation of flower colour was in 3:1 ratio (3 red : 1 yellow).

Qualitative characters in the parental species manifested in the F_1 plant lead to the conclusion that A. lineata was comparatively more closer to the A. cajanifolia than A. albicans.

In the cross between A. albicans x A. cajanifolia, the characters of A. cajanifolia viz., lanceolate shape of first pair of leaves, red standard petal, brown pod colour, red seed coat colour, hairy surface of pod were dominant over those of A. albicans. Shape of the central leaflet, leaf apex and growth habit were observed to be intermediate in the F_1 . F_1 hybrid showed vigour for length and breadth of leaves, number of primary and secondary branches and flower size. In the cross between A. albicans x A. cajanifolia, in F_2 's, standard petal colour segregated into 3:1 ratio (3 red : 1 yellow).

In the cross between A. platycarpa x A. mollis, characters of A. platycarpa, i.e. hairiness of pod and early flowering were dominant over those of A. mollis, but A. mollis showed dominance for seed coat colour. Size of flower and central leaflet shape were intermediate in F_1 hybrid.

In the cross between A. lineata and C. cajan, the characters of Cajanus viz., absence of purple streaks on the standard petal, emerginate leaf apex, deciduous petal, absence of hairs on the pods, non-shattering nature of mature pods, reddish brown seed colour and absence of strophiole were recessive to those of A. lineata (JM 2639). Similarly lanceolate nature of first pair of simple leaves of Cajanus was dominant. Length and breadth of central leaflets, length of petiole, size of standard petal, beak of pod, number of chambers per pod were intermediate in the hybrid. Pod length and length and shape and leaflet shape were nearest to that of A. lineata. Pod colour in C. cajan was green with black streaks and that of A. lineata uniformly green. However, in the hybrid the pods were uniformly reddish brown. Similar observations on morphological characters in C. cajan x A. lineata F₁ hybrid was reported by Kumar *et al.*, (1966), De (1974), Reddy and De (1983). De (1974) reported that in the F₁ hybrids of Cajanus with Atylosia lineata and A. sericea pods were always uniformly reddish brown. In F₂, flower colour segregated into 3:1 (3 yellow with purple streaks: 1 yellow) ratio.

In the cross between A. albicans and Cajanus cajan, characters of Cajanus viz., fugacious stipules, deciduous petals, non-shattering nature of mature pods, brown seed coat colour and absence of strophiole were recessive to those of A. albicans. The shape of central leaflet, leaf apex, length of petiole days to 50% flowering and maturity, thickness of pod, and growth habit were intermediate in F₁ hybrid. Pod colour of Cajanus cajan was green with black streaks and in A. albicans green, the F₁ hybrid showed uniformly reddish brown pods. The F₁ hybrid exceeded to both the parents, in case of leaf and flower size. Similar type

of morphological observations for leaf-shape, pod colour, pod size and growth habit etc. in the F_1 hybrid were also reported by Kumar *et al.*, (1985) and Yadav, (1986) in their studies on C. cajan x A. albicans cross. In F_2 generation on a single branch, leaves with different shapes viz., oval-oblong, obovate and intermediate shape, were seen in some of the F_2 plants. Different types of leaves on a single branch of hybrid of C. cajan x A. albicans is also reported by Kumar *et al.*, (1985). These authors have explained that the variation in leaf morphology accompanied by flowering is a consequence of differential gene expression in different branches and it is likely that this process is temporal event in gene expression. Somatic variation in Cajanus cajan was reported to be chimeral which appeared from seedling stage (Rao and Reddy, 1975). These workers observed that somatic variation could be mutational in origin but mutations have a low probability of occurrence and are not expected to appear simultaneously in several cells of a tissue but the same is not true of treptions which may occur simultaneously in all or several cells of a tissue or regions of the body. Treptions are the result of a natural stimulus which triggers some regulatory process whereas mutations result when a mutagen interferes with the regulatory mechanism of the cell so that they do not work to completion.

In A. scarabaeoides x Cajanus cajan F_1 hybrid, characters viz., hairy leaf surface, persistent petals, red stripes on the standard petals, hairs on mature pods, shattering nature of mature pods, colour of seeds, presence of strophiole were dominant to those of Cajanus cajan. Characters intermediate in F_1 hybrid were leaf shape, leaf apex shape, petiole length, size of flower, length

and breadth of leaf, thickness of pod and growth habit. In F_2 , flower colour segregated into 3:1 (3 yellow with red stripes : 1 yellow) ratio. The present observations in A. scarabaeoides x C. cajan cross are in conformity with those of Roy and De, (1965). These authors also found dominance of most of the characters of A. scarabaeoides in F_1 hybrid alongwith intermediate growth habit. In the present study, pod colour of F_1 hybrid (A. scarabaeoides x C. cajan) was uniformly reddish brown, while pod colour in A. scarabaeoides is green and in Cajanus green with black streaks. In case of flower colour it was noticed that yellow with red striped colour of A. scarabaeoides is dominant over yellow colour of Cajanus cajan and segregated in 3:1 in the F_2 generation. Roy and De (1966) also recorded that purple yellow flower colour of A. scarabaeoides is simple recessive characters to yellow colour of Cajanus and segregated in 3:1 ratio in the F_2 generation. Dominance of red veined standard petal, over yellow colour of standard petal is earlier reported by Tripathi et al., (1984) in their study of cross between A. cajanifolia x C. cajan.

The present study revealed that pod shattering was only to a small extent in A. albicans x A. cajanifolia F_1 plant. But in F_2 generation the plants were found having indehiscent pods. This indicated that pod dehiscence character did not segregate in mendelian ratio as observed by Ladizinsky (1979 b).

The characters like plant height, branching, seed yield per plant etc., behaved quantitatively and in most crosses, degree of dominance ranged from absence to overdominance. Such a phenomenon has also been reported by Sagar and Chandra, (1980) and Nazeen et al., (1983). in the hybrids of lentil.

Segregation of seed coat colour did not follow the mendelian principle. The seed coat colour character showed polygenic inheritance resulting in the appearance of many intermediate seed coat colours. Such type of irregularity in phenotypic appearance of seed coat colours has earlier been reported by Wilson and Hudson (1978a) in lentil. In inheritance of seed coat colours in leguminosae has been thought to be genetical by Harland (1919), Saunders (1959), Anand and Terrie (1964), Bhatta and Terrie (1968), Gorz *et al.*, (1975) and physiological by Kennedy and Cooper (1967), and environmental by Owen (1928).

Reddy *et al.*, (1982) on their studies on 'Genetics of Cajanus x Atylosia' suggested that strophioled and mottled seed characters were governed by inhibitory and complementary gene actions respectively. The hairiness of pods was controlled by a single dominant gene in C. cajan (ICP 6915) x A. scarabaeoides and the glabrous pod character of pigeon pea was inhibited by a gene present in the Atylosia parent. Kumar *et al.*, (1985) also reported that genes controlling seed mottling exhibited complementary interaction while those for seed strophiole and twining habit indicated inhibitory interaction.

For the segregation of lanceolate x obovate leaf shape, in the cross of C. cajan x A. albicans. Kumar *et al.*, (1985) indicated simple mendelian 1:2:1 ratio exhibiting incomplete dominance. Independent assortment of oval x lanceolate leaf shapes is earlier reported by Roy and De (1966) in Cajanus x A. scarabaeoides hybrid and over-oblong x lanceolate leaf shape by Deshmukh and Rekhi (1960) in intervarietal crosses between Cajanus cajan. Deshmukh and Rekhi (1960) also reported dominance of acute apex over round (emarginate) apex in the study of inheritance of leaf in pigeon pea.

Inheritance of red veined yellow standard petal versus yellow standard petal into 3:1 was reported by Dave (1934) in intervarietal cross of Cajanus cajan. Shinde (1971) also reported independent assortment for flower colour in his genetic studies in pigeon peas. Dominance of purple colour of standard petal over yellow one, purple streaked and red streaked over yellow was reported by Ganguli (1967) in Cajanus cajan. In F_2 generation, 1 different plant types with more leafiness were recorded. Usefulness of different plant types is appreciably reported by Tripathi and Patil (1986).

Heterotic vigour in F_1 for flower and leaf size, number of primary and secondary branches was reported by Tripathi et al., 1984 and Tripathi and Patil 1986 in the cross between Atylosia cajanifolia x Cajanus cajan.

Earlier reports have indicated the usefulness of morphological characters in understanding the relationships between the species (Malzew, 1930; Rajhathy, 1960). However, the descriptive morphology alone is not sufficient to fully understand the species relationship and a more precise information based on analysis of chromosome behaviour during meiosis could prove to be more meaningful.

Hybrid : Cytology.

It is now well recognized that during the evolution of species chromosomes undergo changes which make them increasingly non-homologous with ancestral chromosomes and chromosomes of their species descended from the same ancestry. In species hybrids, these changes are reflected according to the degree of divergence attained by the chromosomes of the parents in the form of reducing crossing over and chiasma formation and sterility despite normal

chromosome pairing at metaphase-I. The mechanism of such chromosome evolution, as postulated by Stebbins (1945) is largely the result of structural rearrangements. Many of them are too small to be detected at the later stages of meiosis usually analysed.

In the present investigation, the pairing behaviour of the chromosomes was studied in different hybrids. In A. platycarpa x A. mollis hybrid, entering of all the chromosomes into bivalent association were indicative of good degree of chromosome homology between the two species. The absence of univalents and high pollen fertility further confirmed good homology. Earlier workers also reported normal meiosis in the hybrids of different plant species, followed by high pollen fertility (Krishnaswami et al., 1950; Kid, 1945; Endrizzi, 1957; Magoon, 1964 a, b).

In the hybrids produced i.e. A. albicans x A. cajanifolia, A. lineata x A. cajanifolia, A. lineata x A. albicans, the mean frequency and the percentage of chromosomes involved in different bivalent formation were comparatively lower than those recorded in A. platycarpa x A. mollis. Further in these hybrids, high degree of univalents were also noticed. Precocious separation of 1-6 bivalents was the common feature in A. albicans x A. lineata hybrid. In the present study it was seen that in interspecific crosses between (A. lineata x A. cajanifolia and A. lineata x A. albicans) univalents ranged from 0-16 and in intergeneric crosses (A. lineata x C. cajan, A. albicans x C. cajan, A. scarabaeoides x C. cajan) the univalents ranged from 0-4.

Formation of univalents may be attributed to the precocious separation or desynapsis. Desynapsis is ascribed

to post pachytene separation of paired chromosomes, probably due to failure of crossing over. This can happen only when no chiasma is formed although the chromosome may appear to have paired together. Such apparent pairing of chromosomes is possible because their overall homology has not yet been impaired but they do contain dissimilar segments to the extent to which crossing over between them is not feasible. Thus, sooner a barrier arises to prevent this process, the concentrated chromosomes should be deemed to have diverged in their evolutionary pathways. Ehrenberg (1949), Dobzhansky (1951), Celarier (1955) and Darlington (1957) have also suggested that chiasmata formation is the major factor involved in the desynapsis.

Celarier (1955). is of the opinion that desynapsis may be inherited or may be due to environmental factors which have influence on chiasma formation. Powell (1968) has observed an instance of origin of univalents as a result of some bivalent in the species perityle (compositae). Such bivalents are probably chiasmatic and their chromosomes were only temporarily associated during early stages. A very high percentage of univalents recorded in interspecific hybrids of lotus has been attributed by Grant (1963) to precocious separation of bivalents. Failure of chromosome pairing may well be attributed to non-homology of chromosomes (hybridity).

Genetic control of chromosome pairing has been discussed by Rielly and Law (1965) who have demonstrated that major genes or polygenes determine the extent of synapsis. Gottchalk and John (1964) experimentally raised a desynaptic mutant in Pisum in which chiasma frequency was greatly reduced due to formation of univalents. Beadle (1933) reported some causes of failure of metaphase pairing such as (i) premature chromosome division, (ii)

non specific pairing between homologous chromosomes, (iii) failure of chiasma formation and (iv) breakage of chiasmata and deficient terminal affinity.

Chromosome behaviour at meiosis determines the potentiality of recombination and it depends upon the number of chiasmata per cell and the position and distribution of chiasmata per bivalent. The factors determining these characteristics are under genetic control and are also related to chromosome size (Swanson, 1957; Rees, 1961; Ved Brat, 1965; and Vösa, 1972). In the present study in all the interspecific hybrids chiasma frequency per cell and per bivalent was low as compared to both of the parents involved in hybridization. Low chiasma frequency may possibly be attributed to precocious separation of homologues. Loose pairing in interspecific F_1 hybrids may be due to the reduction in the number of chiasmata. This suggested that even though the chromosomes of the two parents were apparently similar, non-homologous may exist.

High chiasma frequency in intergeneric F_1 hybrids of Cajanus x A. albicans is reported by Dundas et al., (1985). Indirectly presence of high chiasma frequency is also reported by Reddy and De (1983) in C. cajan x A. lineata hybrid, as they found predominance of ring bivalents in F_1 hybrid in comparison to rod bivalents. In the present study in comparison to interspecific hybrids, higher chiasma frequency were recorded in the intergeneric hybrids between Atylosia species and C. cajan. These observations, therefore, suggest that the differentiation in the parental species is primarily at the genetic level which could only be maintained by geographical isolating barriers. There is however the possibility that these species of Atylosia

and Cajanus also be harbouring 'cryptic' structural differences in their chromosome complement as reflected, though indirectly through the variability in mean chiasma frequency in the hybrid. These differences are so small that they cannot be detected cytologically with the resolution of light microscope and reflected by evidences for eg., reduction in chiasmata and/or selective gene elimination of clonal parents.

In the present study high degree of homology between Atylosia and Cajanus was recorded. These observations indicate that the differences in the mean length and arm ratio noted in the large number of parental chromosomes do not constitute major differences between the two genera. The differences in the mean length of chromosomes are circumvented by reciprocal adjustment in lengths in the hybrids resulting in bivalents of intermediate length as in the case of interspecific hybrids between Phaseolus aureus x Phaseolus mungo (De and Krishnan, 1966). Similar observations were also made in the case of intergeneric hybrid between Lycopersicon esculentum x Solanum lycopersicon (Menzel, 1962), and in the hybrid between in P. typhoides x P. purpurium (Pantulu, 1967). The differences in arm ratios of the parental chromosomes are accommodated during pairing in the hybrid in a similar way or by indifferent pairing with respect to the position of centromeres. This may, perhaps be associated by non-homologous pairing near the centromeric region.

In the present study, 1 to 2 heteromorphic bivalents in interspecific hybrids and 1 to 3 in intergeneric hybrids were recorded. Heteromorphic bivalents were also observed by Kumar *et al.*, (1966) in Cajanus x A. lineata; Reddy (1981) in A. cajanus x A. lineata, Cajanus x A. scarabaeoides, Reddy *et al.*, (1983) again confirmed the

frequent presence of two heteromorphic bivalents at metaphase-I of meiosis in Cajanus x A. lineata hybrid. Formation of heteromorphic bivalents was due to possible duplicated segment of chromosomes (Reddy, 1981). Such duplication of chromosome segments has been regarded to give opportunities for the differentiation of genus with new function and the establishment of lateral heterozygosity (Sharma, 1985). The fact that different chromosomes of Cajanus exhibit heteromorphism in the different hybrids of Atylosia suggests that these species have followed separate evolutionary pathways for a considerably period. Similar observation where different chromosomes exhibited heteromorphism in two hybrids (Lycopersicon esculentum x Solanum pennlii) and (L. esculentum x S. lycopersicoides) have been reported by Khush and Rick (1963) and Dana (1966) in Phaseolus aureus x P. trilobus interspecific cross. Kumar (1966) observed that heteromorphism was quite frequent in interspecific hybrids and less in intergeneric hybrids.

Similarly, presence of quadrivalents and trivalents were also reported by Kumar et al., (1966), Reddy (1983), Dundas (1985) in intergeneric hybrids of C. cajan with Atylosia species.

Meiotic anomalies recorded in the present studies were laggards and bridges at anaphase-I and II. Simple bridges may arise due to failure of terminalization of chiasmata as a result of which the chromosomal ends remain sticky in the mid way of the two poles. Presence of chromatid bridge at anaphase-I and II in intergeneric hybrids of Cajanus x Atylosia were reported by many workers viz., Kumar et al., (1966); Reddy (1983) and Pundir et al., 1985.

The most common irregularity was occurrence of micronuclei at sporad stage in all the hybrids. In the present study all the hybrids were semi-fertile except A. platycarpa x A. mollis hybrid where good pollen fertility was recorded. These results by and large suggested that the chromosomal and genetic differences between the genomes of these taxa are expressed by the non-pairing of some chromosomes, resulting in the occurrence of univalents, unequal segregation and lower fertility of the hybrids.

Meiotic abnormalities alongwith infertility are regarded as evidence of hybrid origin of a taxon concerned. Factors like variation in temperature and photoperiod are also known to induce pollen sterility (Jain, 1959; Moss and Heslop Harrison, 1960). Variation in size of fertile pollen grains may be attributed to the unequal separation of chromosomes due to presence of laggards. Thus on the basis of pollen fertility A. lineata appears to be closest to C. cajan followed by A. scarabaeoides and A. albicans.

And, on the basis of univalent frequency recorded in intergeneric hybrid during meiotic studies, A. lineata comes closer to Cajanus cajan followed by A. albicans, A. albicans and A. scarabaeoides (Table-III). This is strengthened by the fact that A. lineata have much similar somatic chromosomes complement. Two heteromorphic bivalents observed during meiotic metaphase may be attributed to the pairing between the chromosomes with and without satellites.

In F_2 generation nearly normal meiosis was noticed with increased pollen fertility as compared to F_1 hybrids. Normalization of meiotic phases and restoration

of pollen fertility may be dependent upon the level of adaptation of a member to a new set of environmental factors.

The somatic chromosome complement of the F_1 hybrid of two haploid sets, are derived from the respective parents. The morphological characters of these two haploid sets of the F_1 hybrid are not different from those of the two parents from which they are derived. The cryptic structural differences between the complements of the two parents have been manifested in the F_1 as evident from the heteromorphic characters of the chromosome pair. Structural differences in the karyotype of intergeneric hybrids are also advocated by Kumar *et al.*, (1966) in Cajanus x A. lineata and Roy and Be (1965) in Cajanus x A. scarabaeoides. These structural differences in the two chromosome sets in the hybrid is supported by the presence of quadrivalents during the reduction division.

Chromosomes of two related species may have some amount of homologous regions which eventually pair and afford chance for the formation of chiasmata. Such chiasmata formation with the homologous region which leads to bivalent formation at metaphase-I and makes 'cryptic' structural changes, between the chromosomes of two genomes and thus, such structural differences in the chromosomes are responsible upto some extent for the observed sterility in the hybrids.

Table - II

Affinities between Alysiola species

| Species | Crossability | F ₁ meiosis | Pollen fertility |
|--------------------------------|---|---|---|
| <u>A. caianifolia</u> | <u>A. platy.</u> x <u>A. mollis</u> | <u>A. platycarpa</u> x <u>A. mollis</u> | <u>A. platy.</u> x <u>A. mollis</u> |
| <u>A. lineata</u> (JM 2639) | <u>A. lineata</u> x <u>A. caianifolia</u> | <u>A. albicans</u> x <u>A. caianifolia</u> | <u>A. albicans</u> x <u>A. caif.</u> |
| <u>A. lineata</u> (JM 3366) | <u>A. albicans</u> x <u>A. caianifolia</u> | <u>A. lineata</u> x <u>A. caianifolia</u> | <u>A. lineata</u> x <u>A. albicans</u> |
| <u>A. scarabaeoides</u> | <u>A. lineata</u> x <u>A. albicans</u> | <u>A. lineata</u> x <u>A. albicans</u> | <u>A. lineata</u> x <u>A. caianifolia</u> |
| <u>A. albicans</u> | | | |
| <u>A. platycarpa</u> | | | |
| <u>A. volubilis</u> | | | |
| <u>A. mollis</u> | | | |

Table - III

Affinities of Atylosia species to Caianne calan

| | Plant Morphology | Karyotype Symmetry | Crossability | F ₁ Meiosis | F ₁ pollen fertility |
|------------|--------------------------------|--------------------------------|--------------------------------|-------------------------|------------------------------------|
| Close ← | <u>A. caianifolia</u> | <u>A. caianifolia</u> | <u>A. lineata</u> (JM 2639) | <u>A. lineata</u> | <u>A. lineata</u> (JM 2639) |
| | <u>A. lineata</u> (JM 2639) | <u>A. lineata</u> (JM 2639) | <u>A. albicans</u> | <u>A. albicans</u> | <u>A. scarabaeoides</u> |
| | <u>A. lineata</u> (JM 3366) | <u>A. lineata</u> (JM 3366) | <u>A. scarabaeoides</u> | <u>A. scarabaeoides</u> | <u>A. albicans</u> |
| | <u>A. scarabaeoides</u> | <u>A. albicans</u> | <u>A. platycarpa</u> | | |
| | <u>A. albicans</u> | <u>A. scarabaeoides</u> | <u>A. volubilis</u> | | |
| | <u>A. platycarpa</u> | <u>A. mollis</u> | <u>A. mollis</u> | | |
| | <u>A. volubilis</u> | <u>A. volubilis</u> | | | |
| | <u>A. mollis</u> | <u>A. platycarpa</u> | | | |
| | | | | | |
| | | | | | |
| Distant. | | | | | |

Induction of polyploidy:

Following the discovery of the use of "colchicine" for the production of polyploids (Eigati and Dastin, 1955), there was considerable enthusiasm amongst the plant breeders who sought to utilize gigas characters of induced polyploids directly in the improvement of plants. There is a small group of material in which the reaction of chromosome doubling is a favourable. Such materials have particularly low chromosome number indicating that the plant concerned are not already either primary or secondary polyploids.

The effects of colchicine on six species of Alyosia ($2n = 2x = 22$) and Calanus calan ($2n = 2x = 22$) were studied and the induced polyploids were evaluated for morphological characters, fertility and cytological behaviour.

In the present study, seed treatment with colchicine was not successful in the production of polyploid plants in any Alyosia species and Calanus calan. The cause of failure appeared to be the drastic effect of the chemical on roots, which failed to produce lateral roots or to show any appreciable development after treatment. Failure in seed treatment is also reported by Bates (1939). Kumar and Abraham (1942) in Phaseolus radiatus, Sen and Chheda (1958) and Siswas and Bhattacharya (1971) in Cyampsis psoraleoides. Higher concentration and increased duration of colchicine treatment reduced the rate of germination in all the species. In C_0 generation the colchicine treated seeds showed delay in germination. The earlier reports on induced polyploids in general, also indicated their late germination (Noquti *et al.*, 1943; Newcomer, 1941; Hoggie, 1946).

To standardize a suitable colchicine treatment method, various variables in procedure can be classified as (i) material i.e., whether dry or soaked seeds or in case of seedlings, the age of seedlings, (ii) strength of colchicine solution, (iii) duration of application and (iv) method of application. Only when precise information is accumulated on the role of these variables, one could expect to get consistent result with particular method. Seedling has the advantage that root system need not be affected and the shoots alone can be treated.

In the present experiments for induction of polyploids, 4-6 days old seedlings were treated by emerging them in a shallow container having different concentrations of colchicine solutions, for the duration of 2, 4 and 6 hours. But seedlings could not survive at increased concentrations and durations. At lower concentrations and short durations, those survived, were found to be diploid after cytological examination. Short duration of the treatment ensures that more than one mitotic cycle is not affected and immersion of seedlings in the colchicine solution ensures that the solution reaches the meristematic tissue and affects the dividing cells. In some of the colchicine treatments, seedlings could not survive. It may be due to the fact that the chromosome number of the cell is suddenly doubled and also because there may be diploid, tetraploid and even a few higher ploid cells in the tissue competing for dominance and division, the metabolism of the treated seedlings will no doubt be in a disturbed state. The similar views for death of seedlings after colchicine treatments in very young age is also advocated by Sikka, *et al.*, (1959) in their studies on induced polyploids in *Trifolium* species.

The method which was found most successful with Alyosia species and Cajanus cajan was seedling treatment where the apical buds were treated with the aqueous solution of colchicine for 8 hours a day for one, two and three days. 0.2% concentration of colchicine was observed to be very effective in inducing polyploidy either in random sectors, branches or whole plant. Success in polyploidy with 0.2% colchicine is also reported by Kumar and Abraham (1942) in Phaseolus radiatus, Bhattacharjee (1956) in Cajanus cajan, Sen and Chadda (1958) in five varieties of black gram, Biswas *et al.*, (1971) in Cyamopsis psoraloides and Jha (1986) in A. scarabaeoides.

In the present study, difference in the percentage success in the induction of polyploidy was noted and it was found that amongst Alyosia species A. platycarpa is most responsive with respect to the production of polyploidy (Table -IV)

Exhibition of highly stunted growth in initial stages was the uniform feature of seedlings survived after colchicine treatment in all the species of Alyosia and Cajanus cajan.

One of the affects generally associated with induction of polyploidy was gigas nature of vegetative as well as floral parts in the polyploids. Such characters though commonly observed, is not, however, a universal commitment of duplication of chromosome number (Chin, 1946; Cozett, 1957; Schertz; 1962; Siddic, 1967).

The induced polyploids showed delayed flowering and maturity which could be due to the changed surface-volume relationship and slower rate of metabolic activities in the tetraploid plants. This slower rate of growth in

polyploids has been attributed to reduced rate of cell division (Wettstein, 1924; Kostoff, 1940; Eigsti, 1947).

In general, tetraploidy is associated with decrease in plant height, thicker stem, lesser number of primary and secondary branches, thicker, broader and greener leaves, bigger flowers, and seeds. An invariable increase in size of stomata and pollen grains is recorded as a most consistent feature. These observations are considered as preliminary indication of the occurrence of polyploidy in the test materials. The morphological data obtained in the present study also indicated that the induced polyploids were more vigorous than their parental forms for certain characters.

The tetraploids of A. lineata and C. caian in C_0 generation had dwarf and bushy appearance in comparison to their diploid counterparts. The inflorescence axis was also shorter with lesser number of flower and pod setting. The pods were shorter with fewer seeds. Increase in the thickness of the stem was observed after the colchicine treatment. This could probably be attributed to the increase in cell size. Morphological variations induced by colchicine treatment were of the similar nature in all the Alysicia species and C. caian. These observations are in conformity with the findings of Sen and Gheda (1958) in five varieties of black gram. (Dwarfing of the treated plants was also reported by Roy and Tapadar (1963) in Rauwolfia and Bose and Panigrahi (1969) in Zinnia)

In the present study the leaves were longer, broader and darker green in the induced tetraploids. Schwanitz (MS) suggested that the dark green colour of the leaves of induced tetraploids are perhaps due to greater thickness of leaves through which light must pass. Levan (1940) reported that tetraploid red clover had larger and

coarser leaves than the diploid plants and in general were of gigas type. Cooper (1938) did not find such gigantism in alfa-alfa tetraploids though the stem, leaves and the individual flowers of each raceme were somewhat larger than those of the diploid plants, the greater size of the flower being most noticeable. Mehta and Swaminathan (1957) in their studies made on Trifolium alexandrinum and Melilotus indica showed that tetraploid berseem plants, as compared to diploid plants, have thicker and longer internodes, longer and thicker petioles, leaves with lower leaf index but greater tendency towards multifoliation (tetra and pentafoliate leaves) and bigger flowers.

The increase in cell size or volume is universally accepted effect of polyploidy and the increase in the size of stomata and pollen grains is the direct instance of this effect. Derman (1940) studied the role of cell size and cell number in producing the ultimate effect of gigantism. This increase was more or less pleiotrophic and resulted in an increase in the size of determinate organs like floral parts and seeds. The present results are in conformity with earlier findings on stomata and pollen size (Kumar and Abraham, 1942; Kumar et al., 1943; Pathak, 1940; Bhattacharji, 1956; Sen and Chheda, 1958; Biswas and Bhattacharya, 1971; Jha, 1986). In the present study a wide variation in the size of pollen was recorded. Levan (1939) has also reported that the pollen grains of tetraploid Petunia were not of uniform size, a greater part consisted of minute deformed pollen grains and large pollen grains usually have four germ pores instead of three.

Induced autotetraploids of all the Alysicarpus species and Cajanus cajan exhibited a fairly good percentage

of fertile pollen grains. In spite of good pollen fertility, the seed setting was comparatively much lower to that of diploid. Schidt and Akerberg (1951) attributed it to the less flower initials and lower cell number while Schwanitz and Schwanitz (1930) recorded less number of ovules per ovary and pollen per anther and increased flower shedding. They attributed all these to lowered surface cell volume ratio.

In the present study, most of the plants showed rudimentary pods with abortive seeds which could not germinate. This development of abortive seeds indicated that some physiological changes in the ovarian tissue might be responsible for this or it may be due to prefertilization upset or post fertilization disturbances leading to abnormal endosperm and embryo development and consequent seed abortion.

Hagoon *et al.*, (1969) found poor seed setting in the induced tetraploid under open pollinated field condition. Similarly in the present study reduced pod and seed setting was recorded in open pollination. However, during selfing, pod setting was much affected which may be attributed to the lack of stigmatic stimulation during selfing as suggested by Mehta *et al.*, (1963).

In C_1 generation, improvement in pod and seed setting, over the C_0 generation was observed in all the species of *Alysicarpus* and *Calanthe calan.* According to Armstrong and Robertson (1956), there might be lack of genic balance in the newly formed tetraploids in C_0 , which would upset the normal operation of allelic interactions and prevent pollen tube growth. In later generation, the adjustment made by selecting from the pool of modifying

factors would make the reproductive process operate smoothly with a consequent improvement in the general level of fertility.

Many workers have attempted to correlate cytological behaviour with seed set. Among the cytological causes of seed sterility it may be mentioned that the inviable/unbalanced gametes resulting from meiotic irregularities forms part of the phenomena as described below:

1. Misedisjunction of multivalents (Darlington, 1937; Kostoft, 1939)
2. Univalents at metaphase-I (Myers and Hills, 1942; 1943; Myers, 1943)
3. Laggards at anaphase-I and II (Sparrow, Ruttle and Nebel, 1942; Myers and Hill, 1942)
4. Spindle abnormalities (Schwanitz, 1948)
5. Non-viability of aneuploids (Lindstrom, 1932; Randolph, 1935; Ramanujam and Joshi, 1941).

In the course of present investigations, the cytological studies in PMC's did not reveal much meiotic irregularities. The quadrivalents formation was comparatively low. Other abnormalities like lagging chromosomes, spindle abnormalities etc. were also of rare occurrence. However, a fairly large number of pentads, hexads, triads, dyads and tetrads were observed for which no clear explanation can yet be given.

Thus it seems that, genetical, cytological, embryological, physiological and environmental factors all contribute towards a reduced seed fertility in autotetraploids.

It is difficult to divide sterility into different components and to ascertain their relative role since all or most of them are interrelated.

Cytology of tetraploids:

An important consequence of induced autotetraploidy is the occurrence of quadrivalents during meiosis. The observations of Morison and Rajhathy (1960) that the multivalent formation is higher in plants with smaller chromosomes than with longer chromosomes. Chromosomes of Alysicis species and Cajanus cajan are small. Due to presence of four homologous chromosomes, multivalent association is expected in all PMCs of autotetraploids. But the formation of only bivalents in many PMCs suggested that the presence of more than two homologous chromosomes is not the only requisite for multivalent formation. In some cells all the chromosomes participated in quadrivalent formation as in A. platycarpa and A. cajanifolia, where 11 quadrivalents were observed and in some cells only univalents were recorded. Most of the cells met with lower quadrivalent frequency. Occasionally associations of more than four chromosomes was noticed. Hence on the basis of these observations, it can be suggested that there must be a genetical control so far as chromosome pairing is concerned. Such reports are available in the literature of (Hiley and Chapman, 1958). In the present study, various types of quadrivalents were noticed, but square shaped quadrivalents were found to be most frequent. Ahloowalia (1968) reported remarkable consistency in the occurrence of the various types of quadrivalents formation from plant to plant in induced tetraploids of rye grass indicating a genetic control of the quadrivalent type formation. He suggested that each set of four chromosomes forms a typical

quadrivalent shape, depending upon chromosome size and chiasma distribution.

In the present investigation of C_0 plants, the mean number of quadrivalents per cell was observed to be 4.6 in Calanus calan, 6.0 in A. albicans, 6.34 in A. volubilis, 4.8 in A. scarebaeoides, 4.7 in A. lineata, 4.31 in A. calanifolia and 5.3 in A. platycarpa which are only 10.4, 13.6, 14.3, 10.9, 10.68, 9.7 and 12.04 per cent, respectively, of the maximum number expected. These quadrivalent frequencies when expressed in percentage are considerably lower than the 2/3rd of the total possible number present. Thus these observations do not satisfy the expectation of Morrison and Rajbathy (1960). In the present study low quadrivalent frequency has been observed. Similar have earlier been reported by many workers foreg. Earnshaw (1942) in Plantago maritima; Kumar et al., (1942) in Calanus calan; Sen and Chheda (1955) in black gram; Bhattacharjee (1956) in Calanus calan; Mehta and Swaminathan (1966) in berseem and Senji; Jha (1986) in Alysicla scarebaeoides.

In the present study average chiasma frequency per cell in the induced tetraploids was slightly lower than twice of those in normal diploids. Theoretically, double the number of chiasmata per cell are expected in the autotetraploids. The general reduction in the mean chiasma frequency per cell recorded is probably due to greater competition in chromosome pairing or precocious terminalization of chiasmata at metaphase-I. However, reduction in chiasma frequency can not be ruled out. Such a reduction in the average chiasma frequency per cell in autotetraploids has also been reported in Brassica (Howard, 1936), Tradescantia (Anderson and Sax, 1936), Secale cereale (Chin, 1946), Primula (Uncott, 1939),

Soybean (Magoon and Tayyab, 1968).

Similar to present observation, univalents at metaphase-I, were also reported by Myers and Hills (1943) in Dactylis glomerata, Bhattacharjii (1956) in Cajanus cajan, Jha (1985) in A. scarabaeoides and they considered these as the most important type of irregularity because of the tendency of such unpaired chromosomes to lag and divide equationally at anaphase-I and to be left in the cytoplasm at telophase-I and II. These univalents were further considered to be an important contributing factor to the formation of aneuploid gametes and micronuclei (Myers, 1943).

A considerable decrease in the frequency of multivalents from C_0 and C_1 was an important feature observed in the present investigation. The average percentage of chromosomes involved in quadrivalent formation in C_1 generation were 7.9 in Cajanus cajan, 10.4 in A. albicans, 9.39 in A. volubilis, 6.9 in A. lineata, 11.2 in A. platycarpa. These results indicated reduction in the percentage of chromosomes associated as quadrivalent in C_1 generation as compared to plant of C_0 generation. These results are in conformity with the earlier reports of changed chromosome behaviour from predominant multivalent formation in maize (Gilles and Randolph, 1951), in Amaranthus (Pal and Khoshoo, 1977) and in cicer Phadnis *et al.*, (1972). This downward trend in quadrivalent frequency might perhaps be due to the effect of selfing which enhanced diploidization as postulated by Magoon and Tayyab (1968) in sorghum. In the two species of Atylosia i.e., A. scarabaeoides and A. cananifolia, there was a slight increase in the frequency of quadrivalent formation in C_1 generation as

11.7 and 11.2 per cent of chromosomes were found to be involved in quadrivalent formation in C_1 generation against 10.90 and 9.7 per cent chromosomes involved in C_2 generation. Similar observation are also reported by Morrison and Rajhathy (1960b) who did not find reduction in quadrivalent frequency in many advanced generations of induced autotetraploids.

In the present investigation, regular separation of chromosomes to the poles was observed at anaphase-I, with infrequent occurrence of laggards with unequal distribution of chromosomes. Darlington (1937) reported that quadrivalents with terminal chiasmata had a more orderly orientation on the equatorial plate and suggested that the simplest and quickest separation at A-I should be among the chromosomes with terminal chiasmata. Myers (1945) found that the quadrivalents with simple rings and chains disjoined more regularly than the quadrivalents of complicated types. Presence of laggards may be attributed to the occurrence of univalents as suggested by Myers (1945) in Lolium perenne. These univalents were further considered to be an important contributory factor to the formation of aneuploid gametes and micronuclei. Meiotic irregularities as laggards and unequal distributions are also reported by Kumar *et al.*, (1945), Pathak (1948), Bhattacharjil (1956) all in Calanus calan and Jha (1986) in A. scarabaeoides.

In the present study, the most important features of induced tetraploids were (1) vigorous for vegetative parts, (2) high pollen fertility, (3) complete tetraploidy, (4) stability of tetraploid in C_1 generation and (5) elimination of aneuploid gametes.

So far as induced tetraploidy in Atylosia species and Caianus caian is concerned, these have not shown much promising results in the present investigation for its economic exploitation except for its luxuriant growth and gigas habit of vegetative parts. Hence tetraploidy in plants where vegetative parts are economically used have comparative good chance of competing with diploids. As in the case of tetraploid berseem and sonji which proved far superior to their diploid counterparts in forage value. (Mehta and Swaminathan, 1955).

In the present studies, observations made in the root tip cells of colchicine treated seeds revealed $4n$, $8n$ and $16n$ ploidy levels at different concentrations and durations in all the species of Atylosia and Caianus caian. These studies were carried out to know the sensitivity of somatic chromosomes towards colchicine and to know the minimum concentration of colchicine required for doubling of chromosomes.

It was observed that among all the Atylosia species and Caianus caian chromosomes of Caianus caian are most responsible towards colchicine followed by A. platycarpa, A. albicans, A. caianifolia, A. volubilis, A. lineata and A. scaphacoides as revealed by percentage of cells showing tetraploidy at the lowest concentration. The minimum concentration of colchicine required for chromosome doubling was found to be 0.05% the highest level of ploidy observed was $16x$. No cell having more than $16x$ ploidy levels could be observed. Ghila, Hussein (1974) reported in Vicia faba, $4n$, $8n$, $16n$ and $32n$ ploidy levels in root tip cells of treated with supersaturated solution of griseofulvin drug for 3 to 120 hours.

Table - IV
response of Atylosia species towards colchicine

| Species | Polyploidy in seed treatment (revealed by mitosis of col. treated seeds) | | Conc./duration of colchicine treatment by which polyploids produced (Seedling treatment) | | % success of treat- ment | Degree of res- ponse towards colchi- cine |
|-------------------------------------|---|-----------------|--|------------------------------------|--------------------------------|--|
| | Minimum conc. | Ploidy level | % of cells | | | |
| <u>Cajanus cajan</u> (SNT coll.) | 0.05% | 4x, 8x | 28.5, 9.58 | 0.2%, 8 hours a day for one day | 1.33 | +++ |
| <u>A. platycarpa</u> | 0.05% | 4x | 19.8 | 0.1%, 8 hrs. a day for 3 days | 3.3 | +++))) |
| | | | | 0.2%, 8 hrs. a day for 3 days | 1.47 | |
| | | | | 0.1%, for 6 hrs. (Immersion) | 2.0 | |
| <u>A. scarabaeoides</u> | 0.05% | 4x | 3.00 | 0.2%, 8 hrs. a day for 3 days | 3.33 | ++ |
| <u>A. lineata</u> (JM 2639) | 0.05% | 4x | 3.12 | 0.2%, 8 hrs. a day for 2 days | 5.0 | +++ |
| <u>A. volubilis</u> | 0.05% | 4x | 4.0 | 0.2%, 8 hrs. a day for 3 days | 3.33 | ++ |
| <u>A. albicans</u> | 0.05% | 4x | 18.8 | 0.2%, 8 hrs. a day for 3 days | 2.85 | ++ |
| <u>A. canifolia</u> | 0.05% | 4x | 6.6 | 0.2%, 8 hrs. a day for 2 days. | 5.0 | +++ |

According to Sharma (1985) colchicine inhibits spindle action and cell wall formation, in this way fusion of cytoplasm of two cells may reveal 8x and 16x ploidy levels. If during the treatment chromosomes are exposed to more than one mitotic cycle or in the prolonged treatment with colchicine, redoubling of chromosomes may occur. Kumar and Abraham (1942) recorded sectorial chimera which showed 8n chromosomes in root tip cells of Phaseolus radiatus. Upto 16x ploidy level is also reported by Landgren (1976) in Pea root protoplast culture.

It is demonstrated by D'Amato (1967) that clearly higher degree of polidy attained by the cells of that species also define the mitotic power of a cell through the maximum degree of polyploidy attained by the cell in presence of colchicine. Evidence for high sensitivity towards colchicine of the young lateral root cells was presented for seedlings of Allium cepa, Pisum sativum, for cuttings of Veronia becabunga (Mangenot, 1939, 1942b) and for seedlings of Vicia faba (Garrigues, 1940). In Vicia faba, Garrigues (1940) found that after colchicine treatment for 72 hours, the highest degree of polyploidy in the main root apex was 16x.

In the present study, maximum number of cells having 4x ploidy level was recorded in the treatment with 0.2% colchicine which is reflected by the fact that complete autotetraploid plants, were found in most of the cases, by the treatment with 0.2% colchicine.

Effects of EMS:

Genetic variability has been induced through mutagenesis in a large number of crops, but the informations available in Cajanus cajan and Alysicia species are very few

(Khan *et al.*, 1973; Khan and Veeraswamy, 1974; Venkateswarlu *et al.*, 1978; Venkateswarulu and Reddy, 1980; Mehetre, 1984). The mutagenesis has been recognised as the most efficient method for induction of morphological and genetical variabilities in the crop plants, especially in those with limited genetic variability (Sigurbjornsson, 1972).

EMS is more and more effective in those very parts of chromosomes where G = bond pairs are in abundance (Freese, 1963). The experimental findings on different test materials by some workers for e.g., Ehrenberg, 1960 in Vicia faba; Swaminathan *et al.*, (1962) in Barley and wheat; Natarajan and Upadhyay (1964) have supported the above facts.

In the present study, the lower doses of EMS treatments brought about slight reduction in seed germination in all the Atylosia spp. and Cajanus cajan. Similar results are also reported by Dubey (1973) in Triticum aestivum Raghuvanshi and Singh (1974) in Trigonella. But at higher doses linear reduction in percentage seed germination was noticed. Similarly, linear reduction in germination percentage of EMS treated seeds is also recorded by Venkateswarlu and Reddy (1980) in Cajanus cajan; Khan *et al.*, (1973, 1974); Mehetre (1984) and Premsekar and Appadurai (1981) in Cajanus cajan. Reduction in seed germination may possibly be due to inhibition of development process in the seeds as a result of the effect of mutagen. According to Brock (1965) reduction in germination is due to induced gross chromosomal breakage. Amar (1968) proposed that endogenous growth regulators play an important role in germination of the seed and there exist a balance in favour of inhibitory substances leading to dormancy.

Selim et al., (1974) while was of the opinion that the reduction in germination percentage of active radicles responsible for seed reduced germination percentage.

Variation in germination may be attributed to genetic make up of the plant concerned. There is an overall disturbance in genetic, cytological and physiological set up of the seeds. The sensitivity of the plant is also one of the factors affecting the germination percentage of a crop.

In the present study, at higher doses of EMS delayed emergence of plumule in the field was one of the observable effects which might be due to slow division rate in meristematic cells of axis. Such mitotic delay has been suggested to be the cause of delayed plumule emergence by Evans et al., (1957), Evan and Scott (1964), Even (1965) and Venkateswarlu and Reddy (1980). He (Venkateswarlu, 1980) observed drastic reduction in germination and survival particularly at the higher doses and opined that different strains of Cajanus differs in their sensitivity reaction to various chemicals and pointed out 0-9.4 per cent plant survival at maturity.

Gaul (1970) observed that the effects of the physical and chemical mutagens, such as physiological damage and gene and chromosomal mutations in the biological material could be measured quantitatively by the degree of reduction in germination, seedling survival of growth and fertility as well as by increase in the frequency of chromosomal aberrations. According to him, the high sensitivity observed in the field for germination, plumule emergence and survival may be attributed to environmental factors which might have greatly enhanced the injuries

caused by mutagenic treatment. The effected seedlings possibly lacked the vigour to come out of the soil surface.

In the present experiment of seed treatment with EMS, it appears that shoots are more chemosensitive than the roots. The differences between chemosensitivity of root and shoot has also been discussed by Avanzi et al., (1966) and Dumanovic and Ehrenberg (1965). It can probably be attributed to their anatomical and physiological differences between their growth mechanism. A great deal of shoot growth is due to the cell elongation, whereas the root growth is more dependent on cell division. In this regard, among 6 species of Alyosia studied, seed germination was most effective in A. cajanifolia and least in A. scarabaeoides. Positively plants of A. scarabaeoides showed maximum survival till maturity and those of A. cajanifolia least. On the other hand, among two strains of Cajanus cajan studied, Cajanus cajan (SNT collection) was found to be more sensitive than Cajanus cajan (ICP 8647).

In M_2 generation, high percentage of seed germination was recorded. This is in agreement with the findings of Sinha and Godward (1972) in lentil and Amer and Hakeems (1964) in Lupinus termis.

In M_1 plants, a gradual reduction was noticed in plant height, number of primary and secondary branches, leaf size, pods/plant, percentage pollen fertility, with the increase in dosage of the mutagens. These findings of the present study are in confirmity with the results of Khan (1974) and Mehetre (1984) in Cajanus cajan. To mention further, Uhlike (1971, 1973), Sharma and Kant (1975)

Sharma and Sharma (1978 a,b and 1979 b,c) advocated that mutagens affects almost every part of plant. At lower dose of EMS treatment, there was not much reduction in plant height as reported by Premsekar and Appadurai (1981). Significant difference in plant height or spread may be because of very high dose used. Premsekar and Appadurai (1981) also reported reduction in number of primary and secondary branches at higher doses. Gradual reduction in different growth and yield characters was earlier reported by Sinha and Godward (1972) and Sharma (1979) in Lentil. Progressively delayed flowering and maturity at higher doses were recorded in Cajanus cajan and all the species of Atylosia, this delay was maximum in Cajanus cajan and minimum in A. platycarpa. Premsekar and Appadurai (1981) observed significant differences between the doses for days to 50% flowering in Cajanus cajan; Sharma (1977) in lentil, Goud et al., (1970) in Sorghum. At lower doses, flowering period was the same as in control. Similar reports are available in Soybean (Patil et al., 1985). Reduction in the number of flower production, delay in flowering and inhibition of flowering at higher doses has been the observable effect of EMS. Likewise, Thakare and Kora (1968) in Citrullus vulgaris, Singh and Gunkel (1965) in Ricinus communis have observed similar effects. Such phenomenon could probably be attributed to mutation in genes having pleiotropic effects.

In the present study, other than trifoliate leaves, unifoliate, bifoliate, quadrifoliate, pentafoilate and leaves with changed or altered phyllotaxy were also recorded in the M_1 plants. Similar variation in leaves was earlier reported by Patil (1985) in Soybean. Pentafoilate condition with numerous variations were also reported by different workers for e.g. Singh et al., (1984) in green gram; Manohar (1985) in Cajanus cajan; Grover (1979) in green gram.

Mutants which exhibit a wider spectrum of phenotypic changes could either be the result of pleiotropic gene action or cryptic chromosome changes. Such new leaf phenotypes brought about by mutagen treatment have been extensively studied in other crops like guar (Mital and Singh, 1970); and jute (Joshua and Rao, 1972).

The observations made in leguminous plants reveal that leaf aberrations are closely related to actual mutation process and are frequently induced due to plasticity of phenotypes (Santos, 1969). It has been suggested that mutagenesis, besides bringing genetic changes also known to affect physiological process directly encountering the destruction of auxin or loss of plant growth regulators.

In the present study abnormal morphological characters noticed in M_1 generation did not segregate in M_2 as shown by Sharma and Sharma (1978a, 1979a and b) for tendrill, leaf and seed coat colour mutations.

Cytology:

The chemicals identified as mutagens represent a wide spectrum with a range of varying biological activities. Phases of cell division affected by chemicals are (i) the stage when the cells enter into division, (ii) the initiation of spindle formation (iii) cytokinesis.

Since DNA synthesis and oxidative phosphorylation are necessary in cell division, mitosis is usually inhibited by chemicals which affect these processes. Chemicals inhibiting the first stage, inhibit successively the division of the cell, the nucleus and the chromosomes. The stage affected is interphase and occasionally early prophase (Sharma, 1985).

The mode of actions of chemicals is variable, though the chromosome component involved is finally DNA. The final upset of the nucleic acid metabolism results in hazards in protein reduplication, causing the chromosomes to break at different loci (Sharma, 1985). Fragmentation followed by translocation may lead to a new pattern of chromosome rearrangements, resulting in heritable phenotypic differences. A working hypothesis presented by Khilman (1971) suggests that DNA is the key substance in chromosome breakage and rejoining and that essentially the same biochemical mechanisms are involved in dark repair of DNA, in genetic recombination and in the formation of chromosomal aberrations.

The chromosomal aberrations produced after mutagenic chemical treatment involved breakage of the chromosomes and later reunions. The unions may occur in original order or a new order following recombinations (Stadler, 1931, Sax, 1940 and Catcheside, 1943). Sax (1940) was of the opinion that the treatment at resting stage produces chromosome breaks. Lea (1946) emphasizes the efficiency of chemicals in causing direct breaks on chromosomes.

The disruption of hydrogen bonds is regarded by some as principally responsible for chromosome breakage (Lawrence *et al.*, 1952; Butler, 1954), whereas others hold that the mode of action is mainly through an effect on sulphhydryl groups (Austin, 1947; 1949a, b; Auerbach, 1952). In chemical treatment susceptibility also varies in different tissues and it has been established that meristematic tissues, being more liable, are susceptible than others.

In the present study, a linear increase in chromosome breakage with increasing concentration was noticed

in all the species of Atylosia and Caianus caian. EMS induced chromosome breaks are also reported in Vicia faba by Ehrenberg (1960), & Rieger and Michalis, (1960); in barley and wheat Nagarjan and Upadhyay, (1964). Translocations are reflected by bridges at anaphase of somatic cell divisions. In the present study single, double and multiple anaphase bridges with or without fragments were observed.

The single bridge arise from break when both chromatids of a chromosomes are broken at the same locus. The dicentric fragment is pulled equally to both side at anaphase and a bridge is formed.

Formation of paired bridge in the mitotic anaphase was described by Sax (1940) and Caldecott and Smith (1952) to be the result of fusion between broken chromosomes rather than broken chromatids.

In the present experiments with EMS, formation of triple and multiple bridges may be attributed to number of chromosomes involved in the breakage and followed by exchanges. Sax (1940) has suggested that when fusion occurs between the broken ends of terminal deletions, a fragment consisting of parts of two chromosomes is the outcome. The relational coiling between the chromatids of the dicentric chromosome persists to metaphase and during the separation at anaphase the dicentric chromatids might be disjuncted easily or form an interlock situation/X-shaped (criss-cross) bridge.

The centric fragments observed in EMS treatments may unite as they possessed centromere and move to either of the poles. Carlson (1938) stated that accentric fragments resulting from breakage of chromosomes tended to be lost from the daughter nuclei due to lack of

centromere, because they move more slowly towards the poles than the normal chromosomes do.

In the present, study, increase in clumping of broken chromosomes was a consistent feature and a gradual increase in clumping of chromosomes was observed with increase in concentration of chemical. The event of clumping is generally met within the plants after irradiation or chemical treatments. (Ghatnekar, 1964; Ohno and Tanikuzi, 1960; Mehra and Mann, 1974).

Stickiness of chromosomes was the pronounced feature as observed at metaphase of somatic cells of EMS treated seeds. Such chromosome stickiness might have been caused due to disbalance at cytochemical level by the secondary effect of chemical treatment. According to Sinha and Godward (1972) fragmentation may often result from a nonspecific manifestation of stickiness which may ultimately causes difficulty in chromosome division and breakage at certain loci. The actual cause of stickiness, whether it is due to mere physical changes or chemical reaction is not known.

Presence of micronuclei in the interphase cells is to be expected as there were many acentric fragments in the metaphases and laggards in the anaphases. Clowes (1964) reported formation of micronuclei a result of exclusion of the acentric fragments of chromosome out of the nuclear membrane during the completion of mitosis. The 'condensed and 'non condensed' daughter nuclei as observed in A. cajanifolia denoted to micronuclei by Shaik and Godward (1972) to the obvious differences between the two groups of micronuclei in structure, thickness of chromatin material and

stainability. The 'non-condensed' micronuclei are formed from several chromosomes or fragments. But the increasing evidences suggesting their ability to divide with the nucleus are furnished by their entering to prophase condition at the same time as the main nucleus. It may be possible that they become 'condensed' after one division and some of the 'condensed' micronuclei that are observed have already passed such a division.

In the present study, meiotic analysis revealed chromosomal configurations such as chain of 3, 4 and 6 chromosomes. In all the Atylosia species and Cajanus cajan, a linear increase in the frequency of rod bivalents with increasing dose was recorded. Reduction in chiasma frequency as shown by increase in the frequency of rod bivalents, at higher doses was registered in the present investigation. Reduction in the number of chiasmata per cell is also reported by Gottschalk and Petrini (1965) in *Pisum sativum*; Mann (1927), Clausen (1931; Godspeed and Avery (1939) in Nicotiana; Riley and Chapman (1966) in wheat; Sinha and Godward (1969) in lentil. Reduction in chiasma frequency may possibly be attributed to the failure of chiasmata formation in both the arms, due to the changes in the nature of genes controlling chiasmata formation.

In the present study, different chromosomal configurations are noted in the flower buds collected from different branches. Variability in chromosomal configurations in different POCs of the same plant or of the same flower bud has been noticed by Gottschalk and Petrini (1965) in pea and Sinha and Godward (1969) in lentil.

In the present study trivalents and univalents were observed in low frequency at lower dosage. The presence of trivalents and univalents in the pollen mother cells could be explained that in such cases either four chromosomes were involved out of which one behaved as univalent or translocation did taken place in only three chromosomes. At lower dose levels, the multivalents were less in number, but at higher dose of chemical treatment there was a sharp increase presumably reflecting the numbers of translocations present. Most of the multivalents recorded at higher dose levels were of open or chain type (adjacent orientation). Occurrence of polyvalent i.e. Chain of 3 or 6 chromosomes has also been reported by Sinha and Godward (1969, 1972), Sinha (1977) in lentil.

The disturb chromosome pairing in diploid PMCs and presence of a large number of univalents in PMCs are indicative of structural changes in the genes controlling chromosomal pairing. Gottschalk and Petrini, 1965; and Riley and Chaoman, 1966).

The presence of giant cells, as observed in C. cajan (SNT collection) was also obtained by Gray and Scholes (1951) in irradiated vicia faba roots. He has suggested the reason of these cells being abnormally large to be their deficiency in nuclear material and consequent inability to divide and form two normal daughter cells. Tolmach and Marcus (1960) also suggested that the giant cells may result from the ultimate failure of the cell division process. The mechanism of giant cell formation, however, has not yet been explained thoroughly.

Formation of 3-distinct chromosomal groups at metaphase-I was noticed in Atylosia platycarpa, A. volubilis, A. lineata. Multipolar spindle formation is a process in which meiotic and mitotic chromosome complements are subdivided into two or more independently functioning groups within the cell at metaphase-I. This process has been described under various terms including incompact spindle (Darlington and Thomas, 1937), 'double plate metaphase' (Huskins, 1948), reductional groupings (Wilson, 1950), multipolar spindle (Therman and Timonen, 1950; Knudson, 1958; Walters, 1958), split spindles (Upcott, 1939; Nielson and Nath, 1961) and complement fractionation (Thompson, 1962). This phenomenon is characterised by the formation of two or more metaphase plates. The consequence of multipolar plates and spindle is some time results in the production of daughter cells with variable chromosome numbers. In plants, this phenomenon has been observed in Oryza sativa (Morinagdad Fukusima, 1934), crested wheat grass, (Agropyron cristatum) (Tal, 1970), mentha (Swanson and Nielson, 1942), and Rubus hybrid (Bammi, 1956; and Walters, 1958).

Swanson and Nielson (1942) while reporting multipolarity in mentha have suggested that certain extra pole determinants of de novo origin are responsible for multipolar spindle formation. Walters (1958) described that the spindle organizers were the same as the pole determinants and suggested that they were compound structures, usually single. These might undergo divisions to give to several super numerary spindle organizers which, in turn, were responsible for extra spindles and multipolarity in cell divisions. Thompson (1962) explained multipolarity in two ways (i) in the first way, chromosomes first group in one plate and then they are brought at different poles (ii) they are brought to their respective places by split spindles.

Tai (1970) explained multipolar meiosis on the basis of genome spindle relationship. According to him, each genome carries its own spindle organizer and movements of chromosomes of a particular genome controlled by its own spindle organizer. So in a species hybrid, where different organizers act as different poles and the chromosomes move to their corresponding organizers resulting in the formation of multipolar spindle. At the same time, Tai (1970) opined that spontaneous or induced breakage of spindle may be another factor leading to multipolar division in many cases.

In the present study, in case of A. caianifolia, it was observed that some time one or two bivalents as well as univalents fail to orient on the equatorial plate at metaphase-I and remain away from the spindle zone. Such bivalent and univalent found in lagging state, while all the normally behaving chromosomes move to the poles. All these situations indicate that even within a cell, different chromosomes may often have different meiotic rhythms showing lack of coordination amongst them. Such situation may be an outcome of genic changes in the chromosomes, due to chemical treatment. Choudhary (1972) have reported several such meiotic abnormalities in brinjal (S. melongena).

At higher doses of treatments, most of the PMCs revealed sticky chromosomes, complex interchanges and unusual configurations and acentric fragments, of left out of the equatorial plate. The complex interchanges might have occurred due to many breakage and reunions and the clumped and unusual configurations were perhaps due to the stickiness, produced in the treated samples.

The single chromatid bridge during meiosis as observed in A. platycarpa and A. scarabaeoides might have

resulted from dicentric chromatids. The appearance of bridge without fragments might be a pointer to the fact that the acentric fragments are involved in the formation of the big, round and dark chromatin mass. Presence of single chromatid bridge was earlier reported by Shaikh and Godward (1972) in L. sativus and V. ervillae. They have also attributed presence of single bridge to the production of dicentric chromatids.

The origin of dicentric bridges and acentric fragments in anaphase-I and II has been ascribed to be a consequence of breakage and reunion of chromatids during meiotic prophase (Haga, 1953; Rees and Thomson, 1955; Lewis and John, 1966; and Newman, 1966) or to be consequence of crossing over between relatively inverted segments (Mc Clintock, 1931). These authors have stated these two phenomenon from observations based on spontaneous breakages of chromosomes since EMS enhances the frequency of chromosomal breaks, it is assumed that these phenomenon might also hold good in the origin of bridges and fragments in anaphase-I and II.

But mostly bridges without fragments have been observed in the present study. Such an absence of fragments may be attributed to their lost during squashing or their taking part in the formation of the micronuclei because the pollen grains in early stages of their development have shown high incidence of such micronuclei. In some cases, the irregular outline of the chromosomes and bridges suggested the possibility of bridge formation by non-separation of chiasmata due to stickiness. Such stickiness might have been caused due to disturbances at cytochemical level by the secondary effect of EMS treatment. It is observed that delayed

Separation of some bivalents was the common feature in all the species of Atylosia and Cajanus cajan. Thus it appeared that due to such stickiness, the separation of the chromosomes were either delayed or completely stopped.

Movement of unequal number of chromosomes as recorded in A. volubilis and A. platycarpa to the poles at anaphase-I and presence of unequal volume of chromatin material in the 4 daughter nuclei after anaphase-II might be the result of occurrence of translocated chain as trivalents or tetravalents at A-I, which causes laggards and unequal segregation of chromosomes. It consequently lead to the formation of unequal pollen grains. Ghatnekar (1964) reported altered number of chromosomes in Vicia faba, and formation of unequal pollen grains in L. sativum was reported by Shakh and Godward (1972).

The unoriented bivalents at metaphase-I and unattached chromosomes at anaphase-I in A. platycarpa as resulted from discrepancies in spindle formation may lead to the unequal distribution of chromosomes at anaphase-I and II. All the cytological abnormalities observed in the present study such as multipolarity, translocated polyvalents, unequal distribution of chromosomes at anaphase-I and II, consequently sometime may lead to formation of more than 4 groups as noticed in A. volubilis and A. lineata. Such unequal distribution of gamete nuclei appeared to go hand in hand with suppression of cytokinesis resulting in the monad, dyads, triads instead of normal tetrads.

In some cases, it was recorded that due to unequal segregation, the unequal tetrad formation to the development of unequal size of pollen grains.

From the result it was apparent that the percentage of non-viable pollen grains appeared to be directly proportional with the increase in dose of EMS treatment. Dose dependence of pollen sterility in EMS treatment is also reported by Nerkar (1977) in Lathyrus. Sterility observed in low chromosomal aberrations in EMS treatment might be attributed to cryptic deletions and specific gene mutations. Fahmy and Fahmy (1957) could demonstrate high ability of alkylating agents to produce deficiencies of cryptic nature in Drosophila melanogaster.

According to Nerkar (1977) chemical mutagens induced pollen sterility, probably due to increased sensitisation of seeds as a result of pre-soaking and decreased intrasomatic selection. Enhanced chemosensitivity caused by presoaking has been attributed to leaching of endogenic protective substances (Kamra et al., 1960), oxygen enrichment (Latteral, 1961), progress of DNA synthesis (Natarajan and Shivashankar, 1965) and changes in the general metabolic condition of the cell (Sharma, 1966). The difference in chemosensitivity among the different species of Alylosia and Cajanus cajan, towards the same strength of chemical mutagen may be due to differences in their genetic set up.

SUMMARY AND CONCLUSIONS

Cajanus cajan is an important pulse crop which includes wild species of Atylosia in its primary gene pool. The productivity of the crop can greatly be improved through introgression of valuable genes from wild species to the cultivated types. Interspecific hybridization is of vital importance in understanding the genome relationships between the species and transferring genes.

Induced polyploidy has been identified as an efficient breeding technique to overcome crossability barrier between species, if any, and create further genetic variability. Tetraploidy in plants, where vegetative parts are economically used have comparatively better chance of competing with diploids. The mutagenesis have been recognised as the most efficient method for induction of morphological and genetic variabilities.

Work done in the light of above facts are summarised as follows:

1. Morphological studies were carried out in seven species of Atylosia viz., A. platycarpa, A. mollis, A. albicans, A. volubilis, A. lineata, A. scarabaeoides, A. cajanifolia and two strains of Cajanus cajan and marked differences were observed. Morphologically, A. cajanifolia was found to be closest to C. cajan.
2. Mitotic and meiotic studies in all the above materials revealed chromosome number $2n = 2x = 22$; $n = 11$. Somatic chromosomes of different Atylosia species and two strains of Cajanus cajan were compared with respect to position of centromeres,

length of short and long arm, L/S arm ratio and T.F. %. No major difference was observed between these taxonomically different genera and species. Similarity lies in all the Atylosia species and Cajanus cajan in possessing secondary constriction in longest chromosome pair. In all the Atylosia species, one pair of satellited chromosomes was observed except in case of A. lineata (JM 3366) and A. volubilis where two pairs of satellited chromosomes were recorded. In case of C. cajan (ICP 8647) two pairs of satellited chromosomes were observed. In C. cajan (SNT collection), no secondary constriction was observed.

3. Interspecific and intergeneric crosses were made to understand the crossability relationship between Atylosia species and Cajanus cajan. On the basis of percentage success of crossability in intergeneric hybridization, A. lineata was found to be closest to Cajanus cajan followed by A. albicans and A. scarabaeoides. With Atylosia volubilis, neither intergeneric, nor interspecific hybrids could be obtained. A. platycarpa, A. volubilis and A. mollis when crossed with C. cajan (as pollen parent) seedless pods were obtained indicating post fertilization barriers in these crosses. Species involved in hybridization and successful hybrids obtained are given in the table A.
4. Important morphological characters of the F_1 hybrids studies at diploid level have been compared with their respective parents. The dominance-recessive relationship between the factor pairs has also been determined in respect of the various qualitative characters. Hybrid vigour for some quantitative characters were obtained in case of A. lineata x A.

caianifolia and A. albicans x A. caianifolia. In intergeneric crosses, most of characters of C. caian viz., colour of standard petal, deciduous nature of standard petal, seed colour, non-shattering nature of mature pods, and absence of strophiole on seed, were found to be recessive to those of Atylosia species. The lanceolate shape of first pair of leaves of C. caian was found to be dominant over ovate shape of first pair of leaves of Atylosia spp.

5. In F_2 generation, all the contrasting characters segregated but inheritance could not be determined because of low population. In F_2 , different plant types were obtained in interspecific as well as intergeneric crosses.
6. Mitotic and meiotic analyses were made in four interspecific and three intergeneric hybrids (Table A). The nature and extent of pairing studied at diakinesis and metaphase-I, and abnormalities like loose pairing, formation of univalents and multivalents at M-I, laggards and bridges at anaphase-I and II were recorded. Pollen formation at sporad stage and pollen fertility percentage at later stages were noticed. Meiotic studies were also carried out in F_2 plants.
7. On the basis of meiotic abnormalities as well as pollen fertility percentage, it is inferred that A. lineata comes closest to C. caian having minimum univalent frequency and highest pollen fertility.
8. All the intergeneric and interspecific hybrids were semi-fertile except A. platycarpa x A. mollis which showed a high percentage of pollen and ovule fertility.

9. The autotetraploidy has been successfully induced in six species of Atylosia and Cajanus cajan (SNT collection) (vide Table B). Success was obtained in apical bud treatment with 0.2% aqueous colchicine solution. No success could be obtained in seed treatment.
10. Detailed mitotic studies were carried out in colchicine treated root tip cells of all the Atylosia species and Cajanus cajan to see the response of somatic cells as well as chromosomes towards colchicine. Different ploidy levels were recorded at different concentrations and durations of treatments. Highest ploidy level (16 n) was observed with 0.2% colchicine solution when used for 6 hours and minimum concentration which brought about chromosome doubling was 0.05 per cent in all the Atylosia species and Cajanus cajan. Quantitative studies revealed that A. platycarpa was most sensitive to colchicine and A. scarabaeoides was found to be least sensitive to this chemical.
11. Detailed morphological and meiotic studies (C_0 and C_1) were made in all the induced tetraploids and compared with respective diploids. Meiotic behaviour and pollen stainability were discussed in relation to plant fertility. Induced tetraploids were more vigorous for certain morphological characters in C_0 as well as in C_1 generation, though associated with reduced seed setting.
12. Meiotic studies in induced tetraploids of A. platycarpa and A. sajanifolia revealed formation of maximum possible quadrivalents (11). Increase in pollen and stomata size was found to be the reliable criteria for judging polyploidy in all Atylosia species and Cajanus cajan.

13. Effect of EMS on six species of Atylosia and two strains of Cajanus cajan were studied (Table B). In the EMS treated materials though the seed germination percentage was good, emergence of plumules in the field was highly effected. A linear reduction in seed germination percentage, number of primary and secondary branches, pods per plant was observed with increase in dose of the chemical.
14. Detailed mitotic analysis in root tip cells of EMS treated seeds was carried out wherein gradual increase in fragmentation and clumping of chromosomes was noticed with increase in dose of the chemical.
15. Meiotic analysis of EMS treated plants revealed bivalent, univalents and polyvalents (chains of chromosomes) at metaphase-I in M_1 plants. Meiotic anomalies included lagards, bridge and delayed separation of bivalents at anaphase-I; lagards at anaphase-II, and at sporad stage dyad, triad, polyad and micronuclei. Pollen fertility was much reduced at higher doses.
16. In M_1 plants, unifoliate, bifoliate, quadrifoliate and pentafoliate leaves with changed phyllotaxy were noticed.

CONCLUSIONS

The differences between Cajanus and Atylosia are those which results purely due to domestication. These include size and vigour of plant and non-shattering character of pod. The gene mutation and selection pressure under domestication underlying the evolution of the cultivated species have probably resulted in accumulation of modifiers and differentiation in the cultivated species. These changes

render unsuccessful seed parent when crossed with Cajanus cajan and thus restricted unwanted recombination in the nature. In the light of present studies, it can be inferred that no change in chromosome number has taken place in the cultivated taxon (C. cajan), while originating from Atylosia species, it appears that structural changes in chromosomes and/or gene mutation might have played a significant role in the evolutionary process.

From the segregating progenies of interspecific and intergeneric hybrids possibility has been explored for selecting better plant types, suitable for dryland as well as rangeland situations.

From the induced tetraploids of Atylosia species plants with more leafiness and other gigas characters can be obtained and used as improved strain on one hand and in developing chromosomal races by crossing them with their diploid progenitors, on the other.

Suitable and useful mutants can be obtained from the segregating progenies of EMS treated plants with good seed setting and pollen fertility, only when large number of progenies are raised.

TABLE A

| Taxa involved in hybridization | Hybrids obtained |
|--------------------------------|--|
| <u>A. platycarpa</u> | <u>Interspecific</u> |
| <u>A. mollis</u> | <u>A. platycarpa</u> x <u>A. mollis</u> |
| <u>A. cajanifolia</u> | <u>A. albicans</u> x <u>A. cajanifolia</u> |
| <u>A. volubilis</u> | <u>A. lineata</u> x <u>A. cajanifolia</u> |
| <u>A. scarabaeoides</u> | <u>A. lineata</u> x <u>A. albicans</u> |
| <u>A. lineata</u> | <u>Intergeneric</u> |
| <u>A. albicans</u> | <u>A. albicans</u> x <u>C. cajan</u> |
| <u>C. cajan</u> (SNT coll.) | <u>A. lineata</u> x <u>C. cajan</u> |
| | <u>A. scarabaeoides</u> x <u>C. cajan</u> |

TABLE B

Name of the taxa and chemicals used in the study

| Name of the chemical | Conc. of chemical | Duration of treatment | Taxa on which the chemical was used |
|-------------------------------|-------------------|--|---|
| Colchicine | 0.025% | 2 to 8 hrs. (seed and seedling treatments) | <u>A. lineata</u> , <u>A. platycarpa</u> , <u>A. volubilis</u> , <u>A. albicans</u> , <u>A. scarabaeoides</u> , <u>A. cajanifolia</u> , <u>C. cajan</u> (SNT collection) |
| Ethyl methane sulfonate (EMS) | 0.2% to 1.0% | 4 and 8 hrs. (seed treatment) | <u>A. lineata</u> , <u>A. platycarpa</u> , <u>A. volubilis</u> , <u>A. albicans</u> , <u>A. scarabaeoides</u> , <u>A. cajanifolia</u> , <u>C. cajan</u> (SNT coll. and <u>C. cajan</u> (ICP 8647) |

R E F E R E N C E

- Ahluwalia, B.S. 1967. Colchicine induced polyploids in rye grass. Euphytica, 16: 49-68.
- Akinola, J.O., A.J. Pritchard, and P.C. Whiteman, 1972. Chromosome number in pigeon pea (Cajanus cajan (L.) Mill sp.) J. Aust. Inst. Agric. Sci., 38: 305-308.
- Ambrose, E.J. and A.R. Gopal Ayenger, 1952. Molecular orientation and chromosome breakage. Symposium on chromosome breakage. Heredity 6 Suppl: 293-298.
- Amer, R.D. 1968. Pot. Rev., 34: 1-13.
- Amer, S. and H. A. Hakeem. 1964. The studies on the effect of Co^{60} gamma radiation on Lupinus termis. Rad. Bot., 4: 95-100
- Anderson, E. and K. Sax. 1936. A. cytological monograph of the American species of Tradescantia. Botan. Gaz., 97: 433-76
- Anand, S.C. and J.H. Torric. 1964. Heritability of frequency and intensity of seed coat mottling and smudgines and interrelationship with other traits of Soybeans. Crop. Sci. 4: 185-186.
- Armstrong, J.M. and R.W. Robertson, 1956. Studies of colchicine - induced tetraploid of Trifolium hybridum L. I. cross and self-fertility and cytological observations. Land. J. Agr. Sci., 36: 21-38.
- Ariyanayagam, R.P. and J.A. Spence. 1978. A possible gene source for early, day length neutral pigeonpeas, Cajanus cajan (L.) Mill sp. Euphytica, 27: 505-509.
- Auerbach, C. 1952. Sensitivity of Drosophila germ cells to Mutagens. Symposium on chromosome breakage. Heredity 6 Suppl. 247-257.
- Avanzi, S.A. Brunori, B. Giorgi. 1966. Radiation response of dry seeds in two variants of Triticum durum. Nut. Res., 3: 426-437.

- Barni, R.K. 1965. Complement fractionation in a natural hybrid of Rubus procerus Muell. and R. laciniatus Willd. Nature, 208: 608
- Baker, J. G. 1876. Flora of British India 2, ed. L. Hocker. London; Reeve. pg. 212-217.
- Babcock, E.B. 1947. The genus grape, I and II Univ. of Calif. Publ. Bot. vols. 21 and 22. 1, 030 pp.
- Beadle, G.W. 1933. Further studies of asynaptic maize. Cytologia, 4: 269-287.
- Bhatta, G.M. and J.H. Torric. 1968. Inheritance of pigment colour in the soybean. Crop. Sci. 8: 617-619.
- Bhattacharjee, S.K. 1956. Study of autotetraploid cajan (Linn.) Mill sp. Caryologia, 9: 149-159.
- Biswas, A.K. and N.K. Bhattacharyya. 1971. Induced polyploidy in legumes. I. cyamopsis psoraloides DC. cytologia, 36 (3): 469-479.
- Rose, S. 1957. Aberrations in the nucleolar chromosomes of inbred Rye III. Size variation in inbred lines and population plants. Hereditas, 43: 621-643.
- Rose, S. and U.C. Panigrahi., 1969. Studies on induced polyploidy in Zinnia linearis Benlth. Cytologia, 34 (1): 103-111.
- Brock, R.D. 1965. Rad. Bot., 5: 543-555.
- Butler, J.A.V. 1954. The action of carcinogenic agents and radiations - some implications of new ideas of the structure of desoxyribonucleic acid. Acta, 10: 97-98.
- Buhashad, T.J. 1956. The crossing of beans (Phaseolus spp.) Euphytica, 5: 41-60
- Carlson, J.G. 1938. Mitotic behaviour of induced fragments lacking spindle attachments in the neuroblasts of the grasshopper. Proc. Natl. Acad. Sci., (U.S.) 24: 500-507.

- Catcheside, D.G. 1984. Genetic effects of radiation. Advances in Genetics, 2: 271-358. Academic press. Inc., N.Y.
- Caldecott, R.S. and Smith, L. 1952. A study of X-ray induced chromosomal + anomalies in barley - Cytologia, 17: 224-242.
- Chenneveerai and D.G. Krishnappa. 1968. Desynapsis and sterility in Solanum wendlandii. Hook. Cytologia, 33: 151-154.
- Chaturvedi, S.N. and R.P. Sharma, 1978. EMS induced sterile mutants in redgram. Curr. Sci., 47 (5): 173-174.
- Chin, T.C. (1946). The cytology of polyploid Sorghum. Amer. J. Bot., 33: 611-614.
- Clower, F.A.L. 1964. Micronuclei and radiosensitivity in the root meristem of Vicia faba. Ann. of Bot., Ann. of Bot., 28 (110): 345-350.
- Clausen, R.E. 1931. Inheritance in Nicotiana tobacum XI The fluted assemblage. Amer. Nat., 65: 316-331.
- Cleland, R.E. 1963. Adv. Genetics, II. 147.
- Cooper, D.C. 1938. Artificial induction of polyploidy in Nicotiana. Amer. Nat., 75: 291-309.
- Dave, B.B. 1934. Inheritance of characters in Cajanus indicus. Ind. J. Agric. Sci., 4: 674-691.
- Dana, S. 1964. Interspecific cross between tetraploid phaseolous species and P. ricciar dianus Ten. Nucleus, 7: 1-10.
- Dana, S. 1965. Phaseolus aureus (Roxb) X Tetraploid Phaseolus species cross. Revista de Biologia 5 (1-2) : 109-114.
- Davidson D. 1965. A differential response to colchicine of meristems roots of Vicia faba. Annals of Botany, N.S. 29: 253-264.

- Tal, W. 1970. Multipolar Meiosis in diploid crested wheat grass, Agropyron Cristatum. Amer. J. Bot., 57 (10): 1160-1169.
- Therman, E. and S. Timonen. 1950. Multipolar spindles in human cancer cells. Hereditas, 36: 393-405.
- Thakare and Bora, 1960. Curr. Sci., 29: 8.
- Thompson, Maxine. M. 1962. Cytogenetics of Rubus III. Meiotic instability in some higher polyploids Amer. Jr. Bot., 49: 575-582.
- Tolmach, L.J. and P.I. Marcus. 1960. Development of X-ray induced giant Hela cells. Exptl. Cell Res. 20: 350-360.
- Tripathi, S.N. B.D. Patil and G.P. Shukla, 1984. Phylogenic and hybridization potential in Atylosia and Cajanus species. Forage Res. 10: 5-9.
- Tripathi, S.N. and B.D. Patil, 1984. Interspecific cross between Atylosia albicans and Atylosia scarabaeoides. Curr. Sci., 53: (14), 775-777.
- Tripathi, S.N. and B.D. Patil, 1986. Morphogenetic studies in Atylosia cajanifolia x Cajanus cajan forage Research. 12(1): 49-52.
- Tripathi, S.N. 1986. Cytomorphological studies in Cajanus cajan x (Atylosia cajanifolia x Atylosia scarabaeoides). J.I.S. (suppl.) 65:77
- Tripathi, S.N. and B.D. Patil. 1986. Trispecific cross in the genus Atylosia, Cytologia, (In press).
- Tripathi, S.N. 1987. Hybridization of Cajanus cajan with Atylosia spp. Nat. Symp. on Cytogenetic Researches in India, Feb. 21-23, PP.69.
- Uhlick, J. 1971. Mutational efficiency of thermal neutrons in Lens esculenta Biol. Plantarum, 13: 216-233.

- Uhlick, J. 1973. Comparison of Mutagenic activity of N. ethyl-N-Nitroso urea and N-methyl-N-Nitroso-guanidino in Lens esculenta, Biol. Plantarum, 15, 274-279.
- Upcott, A. 1939. The nature of tetraploidy in Primula Kewensis. J. Genet., 39: 79-100.
- Varrana, A. 1949. Spindle abnormalities and variation in chromosome number in Ribes nigrum. Hereditas, 35: 136-162.
- Vander Maesen, L.J.G. 1980. Taxonomy of Cajanus In, International workshop on Pigeonpeas. ICRISAT, 2: 10.
- Ved Brat, S. 1965. Genetic system in Allium - III. Meiosis and breeding systems, Heredity, 20: 325.
- Veeraswamy, R., N.M. Sherief, 1973. Artificial crossing in Cajanus cajan. Madras Agri. Jr., 60: 1826-1827.
- Venkateswarlu, S., R.M. Singh, and R.B. Singh. 1976. EMS-induced Multicarpellate condition in Cajanus cajan. Curr. Sci., 45 (2): 773-774.
- Venkateswarlu, S., Singh. R.M., Singh. R.B., and Singh B.D. 1978. Radio sensitivity and frequency of chlorophyll Mutations in Pigeon pea. Ind. Jr. Genet., 38: 90-95.
- Venkateswarlu, S., R.M. Singh and L.J. Reddy, 1980. Induced mutagenesis in pigeonpea with Gamma Rays, Ethyl Methane sulfonate (EMS) and Hydroxylamine (HA), Int. Work. Pigeon pea. ICRISAT, 2: 67-74.
- Vosa, L.G. 1972. Two track heredity : differentiation of male and female Meiosis in Tulbaghia, Caryologia, 25: 275.
- Walters, M.S. 1950. Spontaneous breakage and reunion on Meiotic chromosomes in the hybrid Bromus tritici X B. Maritimus. Genetics, 35: 11-37.

- Walters, Marta S. 1958. Aberrant chromosome movement and spindle formation in meiosis of Bromus hybrids. An interpretation of spindle organization. Amer. Jr. Bot. 95 : 271-289.
- Wettstein, F.V. 1924. Morphologie and Physiologie des Formwechsels der Moose auf genetischer Grundlage. Z. Indukv. Abstamm of u. Vererbgslehre., 33: 1-234.
- Wight, R. and C.A. Walker - Arnoot 1834. Prodrromus florum Peninsulae Indiae orientalis. London 1, 256.
- Wilson, G.B. 1950. Cytological effects of some centrioles J. Hered., 41: 227-231.
- Wilson, V.E. and L.W. Hudson. 1978 a. Seed coat colour anomalies in early generations of lentils. J. Hered., 69: 205-206.
- Wilson, V.H. and A.G. Law. 1972. Natural crossing in Lens esculenta. Moench. J. Amer. Hort. Sci., 97: 142-143.
- Yadav, T.S. 1986. Cytomorphological study of F_1 hybrids of Cajanus cajan x Atylosia albicans. Jr. I.B.S. 9th All India Botanical Conference, 65: 28-30 Dec. PP. 77.

.....

- D. Anato Francisco and vittoria Nuti Ronchi, 1967. The response to colchicine of meristems of roots of Vicia faba. Caryologia, 21: 53-64.
- Darlington, C.D. 1937. Recent advances in cytology. Blakiston. Philadelphia.
- Darlington, C.D. and P.T. Thomas. 1937. The breakdown of cell division. in restuca lolium derivatives Ann. Bot., 1: 747-762.
- Darlington, C.D. 1963. Chromosome botany and the origin of cultivated plants. Hafner publishing Co. New York and George Allen and Unwin. London.
- Darlington, C.D. 1965. Cytology. J. and A. Churchill, London.
- Dobzhansky, Th. 1951. Genetics and the origin of species, third edn. Columbia Univ. Press New York.
- De candolle, A.P. 1813. Catalogus Hortus Monspeliensis; 85-86.
- Delaunay, L. 1926. Phylogenetische chromosome in Verkürzung. Zeitschr. Zellf u. Mikr. Anat., 4: 338-364.
- Dersen, H. 1940. Colchicine polyploidy and technique. Bot. Rev., 6: 599-635.
- Deodikar, G.B. and C.V. Thakar, 1956. Cytotaxonomic evidence for the affinity between Cajanus indicus spreng. and certain erect species of Atylosia W. & A. Proceedings, Indian Academy of Sciences 43 (B): 37-45.
- Deshmukh, N.Y., and S.S. Rekhi. 1960. Inheritance of leaf in Pigeon pea (Cajanus cajan (L) Mill sp). Curr. Sci., 29: 237-239.
- De D.N., 1974. Pigeon pea. In: Hutchinson JB (ed) Evolutionary studies on world crops. Cambridge University Press, Cambridge, pp 79-87.
- De, D.N. and Krishnan R. 1956. Cytological studies of the hybrid Phaseolus aureus x P. mungo. Genetica, 37: 588-600.

- Dogett, H. (1957). Tetraploid varieties of S. vulgare.
Nature, Lond., 179: 786.
- Dustin, P. 1947. Some new aspects of mitotic poisoning.
Nature, 159, 794-797.
- Dustin, P. 1949a. Mitotic poisoning at metaphase and - SH
proteins. Proc. 7th Intern. Congr. Exptl. Cytol.
(1949). Exp. cell Research (Suppl.) 1, 153-155.
- Dustin, P. 1949 b, Les Poisons mitotiques et le mode d'action
des radiation ionisantes en biologie, Ruclides
(Madrid) 9, 188-195.
- Dumanoric, J. and L. Ehrenberg, 1965. Growth inhibition
in cereal seedlings induced by gamma radiation at
different oxygen tensions. Rad. Bot., 5, 307-319.
- Dubey, S.D. 1973. Mutation studies in bread wheat (Triticum
aestivum) M. Sc. (Genetics) thesis, I.A.R.I. New Delhi.
- Dundas, I.S., E.J. Britten, D.E. Byth and G.H. Gordon, 1985.
5th SABRAO Congress, Bangkok, Nov. 25-29.
- Earnshaw, F. 1942. Experimental taxonomy. V cytological
studies in sea plantains allied to Plantago
Maritima L. New Phytol., 41: 151-164.
- Ehrenberg, B.C. 1960. Quoted in localized chromosomal
breakage induced by EMS and hydroxyl amine in V. faba.
Chromosome, (Berl.) 15: 156-169.
- Ehrenberg, B.C. 1949. Studies on asynapsis in the genus
Ulmus glabara - Huds. Hereditas, 35: 1-26.
- Eigsti, O.J. 1947. The pollen tube method for making
comparisons of differences in mitotic rates between
diploid and tetraploid. Genetics, 32: 85.
- Eigsti, O.J. and P.J. Dustin, 1955. Colchicine in
agriculture, Medicine, Biology and chemistry. Iowa
state college press, Ames, Iowa.

- Endrissai, J.K. 1957. Cytological studies of some species and hybrids in the Su - sorghums, Bot. Gaz., 119: 1-10.
- Evans, H.J., G.J. Neary and S.M. Tolkinson. 1957. The use of colchicine as an indicator of mitotic rates in broad bean root Meristem, J. Genetics, 55: 487-502.
- Evans, H. J. and D. Scott. 1964. Influence of DNA synthesis on production of chromatid aberrations by X-rays and Maleic hydrazine in vicia faba. Genetics, 49: 17-38.
- Evans, H.J. 1965. Effects of radiation on Meristematic cells. Rad. Bot., 5: 171-182.
- Fahmy, O.G. and M.J. Fahmy 1957. Comparison of chemically and x-ray induced mutations in Drosophila Melanogaster. Advan. Radiobiol., 437-47.
- Frahm - Leliveld, J.A. 1965. Cytological data on some tropical vicia species and cultivars from cowpea asparagus bean. Euphytica, 14: 251-270.
- Freese, E. 1963. Molecular Mechanism of Mutations. Molecular genetics part I. Ed. J.H. Taylor. P. 207-270. New York and London Academic Press.
- Gaul, H. 1970. Mutagen effects observable in the first generation. I. Plant injury and lethality. II. Cytological effects. III. Sterility manual on Mutation breeding pp. 85-99 (Tech. Rept. Ser. No. 119) International Atomic Energy Agency, Vienna.
- Ghosh, B.N. 1950. Physiological studies on the effect colchicine on rice II. Proc. Nat. Inst. Sci. India, 16: 135-145.
- Ghatnekar, M.V. 1964. Primary effects of different mutagens and the disturbances induced in the meiosis of X_1 and X_2 of vicia faba. Caryologica, 17: 219-244.
- Ghosh, M. 1964. A study of Karyotypes of different varieties of Oryza with reference to phylogenetic interrelationships. Nucleus, 7: 77-92.

- Gilles, A. and Randolph, L.F. 1951. Reduction of quadri-valent frequency in autotetraploid Maize during a period of ten years. Amer. Jr. Bot., 38: 12-17.
- Good speed, T.H. and Avery. 1939. Trisomics and other types of Nicotiana sylvestris. J. Genet., 38: 381-458.
- Gottschalk, W. and R. Willalobospetrini. 1965. The influence of Mutant genes on chiasmata formation in Pisum sativum. Cytologia., 30: 88-97.
- Goud, J.V., K.M.D. Nayer and M.G. Rao. 1970. Mutagenesis in Borghum. Ind. Jr. Genet., 30: 80
- Gorz, J.J., J.E. Specht and F.A. Haskins. 1975. Inheritance of seed and seedling colour in sweet clover. Crop. Sci., 233-238.
- Grant, W.F. 1963. Desynapsis in Lotus hybrid Proc. XI Int. Genet. Congr. (Abs.) In Genetics Today, 1: 132-133.
- Gray, L.H. and M.E. Scholes. 1951. The effect of ionizing radiations on the broad bean root, Part 8, Growth rate studies and histological analysis. Brit. J. Radiol., 24 (279): 176-180.
- Grover, I.S. and S.K. Tejpal. 1979. Genetica, Polon., 20(4): 529.
- Gurdon, S.A. 1954. Occurrence formation and inactivation of auxin A. Rev. pl. physiol. 5: 341-388.
- Haga, T. 1953. Meiosis in Paris. 11. Spontaneous breakage and fusion of chromosomes. Cytologia, 18: 50-66.
- Harland, S.C. 1919. Inheritance of certain characters in cowpea (Vigna sinensis) II. J. Genet., 1: 193-205.
- Hooker, J.D. 1875. Flora of British India, vol. 2, 215 pp.
- Hooker, J.D. 1876. The flora of British India, vol. II Delhi and Dehradun, India; BSMPS and Periodical Experts.
- Hooker, J.D. and B.D. Jackson, 1895. Indokewensis plantarum phanerogor Marum, 1: 249.

- Honna, S. and O. Heecht. 1958. Bean inter specific hybrid involving phaseolus coccineus x P. lunatus. Proc. Amer. Soc. Hort. Sci., 75: 360-365.
- Howard, H.W. 1936. The cytology of autotetraploid kale, Brassica oleracea, Cytologia, 10: 77-87.
- Huskins C.L. 1948. segregation and reduction in somatic tissue I. Initial observation on Allium cepa. J. Hered., 39: 311-325.
- Muziwara, Y. 1962. Karyotypic analysis in some genera of compositae VIII. Further studies in the chromosome of Aster. Amer. Jr. Bot., 49: 116-119.
- Mussien, M.G. 1974. C. Mitotic effect of griseofuloin Bull. fac. Sci. Cairo Univ., 0 (47): 99-116.
- Jain, S.K. 1959. Male sterility in flowering plants. Biblio. Genet., 18: 101-106.
- Jagthesan, D. and M.J. Ratnambal. 1969. Karyotype analysis in saccharum robustum. Nucleus, 12: 23-30.
- Jha, R.P and H.C. Jha 1986. Studies on colchicine induced tetraploid of Atylosia scarabaeoides. Ind. Jr. Bot. Soc., 65 (2): 150-157.
- Joshua, D. and C.Rao. 1972. Ind. Jr. Genet., Pl. Breed., 32: 292-299.
- Joseph, L.S., J.C. Pouwlemp. 1978. Karyomorphology of several species of Phaseolus and vigna cytologia, 43: 596-600.
- Kanra, O.P., S.K. Kanra, R.A. Nilam and C.F. Konzak. 1960. The radiation response of soaked barley seeds. II. Hereditas., 46: 261-273.
- Kennedy, B.W. and R.L. Cooper. 1967. Association of virus infection with mottling of soybean seed coats. Phytopathology. 57: 35-37.

- Khan, W.M.A., N. Sivaswamy and K.R. Ramaswamy. 1973. 1973. sensitivity of the red gram C. cajan (L.) Mill sp.) strains to different Mutagens. Madras Agric. J., 60 (6): 406-407.
- Khan, Muhammed Ali, W., and R. Veeraswamy. 1974. Mutations induced in red gram (Cajanus cajan (L.) Mill sp.) by gamma radiation and EMS. Radiation Botany, 14: 237-242.
- Kidd, H.J. 1956. The morphology of the panicle in the cultivated sorghums. Ph. D. dissertation, Washington Univ., St. Louis.
- Knudson, O. 1958. Multipolar spindle in seminal epithelium of sterile bulls. Ark. Zool. 11: 119.
- Kostoff, D. 1939. Evolutionary significance of chromosome length and chromosome number in plants. Biodynamica, 51: 1-14.
- Kostoff, D. 1940. The frequency of cell divisions in polyploid plants. Curr. Sci., 9: 217-28.
- Kostoff, D. 1943. Cytogenetics of the genus Nicotiana. states Print House, Sofia, P. 1073.
- Krishnaswamy, N. and A. Rangaswamy. 1935. Chromosome number in Cajanus indicus. Curr. Sci. 3(12): 614.
- Krishnaswamy, N., V.S. Raman, and P. Chandrasekharan. 1956. An interspecific hybrid of grain Sorghum and Johnson grass - S. halepense (2n = 20) x S. roxburghii (2n = 20) Curr. Sci., 25: 195-197.
- Kumar, L.S.S. and A. Abraham. 1942. Induction of polyploidy in crop plants. Curr. Sci. 2: 112-113.
- Kumar, L.S.S., A. Abraham and V.K. Srinivasan. 1945. Preliminary note on autotetraploidy in Cajanus indicus Spreng. Prog. Indian Acad. Sci. (Sec. B) 21: 301-306.
- Kumar, L.S.S. and Thombre, M.V. 1958. An intergeneric hybrid of Cajanus cajan (L.) Mill sp. x Atylosia lineata W. & A. Journal of the University of Poona, 12: 13-16.

(x)

- Kumar, L.S.S., N. V. Thombre and R. D'cruz, 1958. Cytological studies on an intergeneric hybrid of Cajanus cajan and Atylosia lineata. Proceedings, Indian Academy of Sciences, 47 (B): 252-262.
- Kumar, P.S., N.C. Subrahmanyam and D.G. Paris. 1985. Morphological variation and inheritance in a Pigeon pea intergeneric hybrid. Cum. Sci., 34(7): 346-348.
- Kumar, P.S., N.C. Subrahmanyam and D.G. Paris 1985. Intergeneric hybridization in pigeonpea. I. Effect of hormone treatments. field crop Research, 10: 315-322.
- Latteral, R.L. 1961. The influence of Oxygen on the radiosensitivity of Maize chromosomes during seed germination. Rad. Res., 14: 480-485.
- Landgren C.R. 1976. Patterns of Mitosis and differentiation in cells derived from Pea root protoplasts. Amer. Jr. Bot., 63 (4): 473-480.
- Lackey, J.A. 1977. A. Synopsis of Phaseolae (Leguminosae : Papilionoideae). Ph. D. Thesis, Iowa State, University, Ames, Iowa, USA, 293 PP.
- Ladizinsky. 1979 b. The genetics of several morphological traits in the lentil. Journ. Hered., 70: 135-137.
- Levitaky, G.A. 1924. The material basis of heridity. Kiev, State Publ. Office of the Ukraine, 166 PP.
- Levitaky, G.A. 1931. The morphology of Chromosomes. Bull. Appl. Bot. Genet. Plant Breed., 27: 19-174.
- Levan, A. 1931. Cytological studies in Allium. A. Preliminary note. Hereditas, 15: 347-356.
- Levan, A. 1932. Cytological studies in Allium I. chromosome morphological contributions. Hereditas, 16: 257-294.
- Levan, A. 1934. Cytological studies in Allium IV. Allium Matranthum. Hereditas, 18: 349-359.

- Levan, A. 1935 a. Cytological studies in Allium VI. Hereditas, 20: 289-330.
- Levan, A. 1935 b. zytoloische studies in Allium schoenoprasum. Hereditas, 22: 1-28.
- Levan, A. 1939. Tetraploidy and Octoploidy induced by colchicine in diploid Petunia Hereditas, 25: 109-131.
- Levan A. 1940. Production of tetraploid red clover. Sverig. Vetenskapsakad. Tidskr., 50: 115-124.
- Lee, D.E. 1946. "Actions of Radiations on Living cells" Cambridge University Press. London.
- Lindstrom, E.W. 1932. A fertile tetraploid tomato. Jour. Hered., 23: 115-122.
- Lorz, A.P. 1952. An interspecific cross involving the lima bean, Phaseolus lunatus L. Science, 115: 702-703.
- Mann. 1927. Quoted in "The influence of Mutant genes on chiasma formation in Pisum sativum. Cytologia, 30: 88-89.
- Manton, I. 1932. Introduction to the general cytology of the cruciferae. Ann. Bot., 46: 509-556.
- Malshev, A.I. 1930. Wild and cultivated oats (Poa griseb.). Bull. Appl. Bot. Gen. and Pl. Breed. suppl., 38. Leningrad. 473-506.
- Mather, K. 1932. Chromosome variation in crocus I. Jour. Genet., 26: 129-142.
- Mangenot G. 1936. Ebauches radicellaires et colchicine. C.R. Acad. Sci., 208: 1105.
- Mackery, I. 1951. Neutron and X-ray experiments in barley. Hereditas, 37: 421-464.
- Manohar Rao, D. and Tumala P. Reddy, 1985. Induction of morphological mutants in Cajanus cajan (L.) Mill sp. Ind. J. Bot., 8 (2): 198-202.

- Magoon, M.L., S. Ramanujan and D.C. Cooper 1962. Cytogenetical studies in relation to the origin and differentiation of species in the genus solanum L. Caryologia, 15(1) : 51-252.
- Magoon, M.L. 1964 a. Review of fundamental work in the field of sorghum cytogenetics; the role of some internal mechanisms in speciation in the genus sorghum. Prog. 3rd All Ind. Millet conf., Coimbatore, India.
- Magoon, M.L. 1964 b. The role of some internal mechanisms in species differentiation. A. Chapter in Plant Breeding Research at I.A.R.I.
- Magoon, M.L. and M.A. Tayyab. 1968. Studies on induced polyploids in the genus sorghum. Nucleus, 11(1): 19-133.
- McClintock, B. 1931. Cytological observations of deficiencies involving known genes, translocation and an inversion in Zea mays. Missouri Agr. Exp. Sta. Research Bull. 163: 1-30.
- Mehata, R.K. and M.S. Swaminathan, 1957. Studies on induced polyploids in forage crops. Ind. J. Genet. Pl. Breed. 17: 27-57.
- Menzel, M.Y. 1962. Pachytene chromosomes of the inter-generic hybrid Lycopersicon esculentum x Solanum lycopersicoides. Amer. J. Bot., 49: 605-615.
- Mehra, P.N. and C. P. Malik. 1963. Cytology of some Indian chamopodiaceae. Caryologia, 16: 67-84.
- Mehra, R.K. and M.S. Swaminathan 1957. Studies on induced polyploids of forage crops. I. Survey of previous work. Ind. J. Genet., 17: 27-57.
- Mehra R.K., K.N. Subramanyam and M. S. Swaminathan, 1963. Studies on induced polyploids in forage crops III. Growth, Cytological behaviour and seed fertility of C₁, C₂ and C₃ cultures of Berseem. Ind. J. Genet. Pl. Breed 23: 67-81.

- Mehra, P.N. and S.K. Mann. 1974. Cytogenetical effects of chemical Mutagens on Pterotheca falconeri I. Monofunctional alkylating agents. Nucleus, 17: 167-182.
- Mehetre, R.B., Deshmukh and R.G. Rodege. 1984. Effect of gamma rays on different growth and economic characters in Pigeon pea. 2nd. Jk. Heridity, XIII (1-4): 19-23.
- Mikaelson, K. 1968. Effect of fast neutrons on seedlings growth and Metabolism in Neutron irradiation of seeds II. pp. 63-70. Tech. Rept. sr. No. 42. IAEA Vienna.
- Mital S.P. and Singh, H.B. 1970. ICAR., New Delhi, 188-200.
- Morinagaad Fukuslima. 1934. Quoted in Multipolar Meiosis in diploid crested wheat grass, "Agropyron Cristatum" Amel. J. Bot., 57 (10) : 1160-69.
- Morrison, J.W. and T. Rajhathy 1960. Frequency of Quadri-valents in autotetraploid plants. Nature Lond, 187: 528-530
- Morrison, J.W. and T. Rajhathy. 1960 b. Chromosome behaviour in autotetraploid cereals and grasses. Chromosoma, (Berlin) 11: 297-309.
- Moss, G.I. and J. Heslop - Harrison. 1960. Photoperiod and pollen sterility in Maize. Ann. Bot., (N.S.) 32: 833-848.
- Mohamed sheriff, N. and R. Veeraswamy. 1977. Genotypic and phenotypic variability of Mutants in red gram (Cajanus cajan (L.) Mill sp.). Madras Agric. J., 64(1): 44-45.
- Mukhopadhyay, S. 1986. Intergeneric relationship between three genera of Leguminosae. J.L.R.S., 65(1): 124-129.
- Myers, W.M. and H.D. Hill. 1942. Variation in chromosomal associations and behaviour during and meiosis among plants from open pollinated populations of Pactylis glomerata. Bot. Gaz., 104: 171-177.

- Myers, W.M. 1943. Analysis of variance and covariance of chromosomal associations and behaviour during meiosis in clones of pactylis glomerata L. Bot. Gaz., 104: 541-552.
- Myers, W.M. and H.D. Hill 1943. Increased Meiotic irregularities accompanying inbreeding of pactylis glomerata L. Genetics, 28: 383-397.
- Myers, W.M. 1945. Meiosis in autotetraploid Lolium perenne in relation to chromosomal behaviour in autopolyploids. Bot. Gaz., 106: 304-16.
- Navashin, M. 1926. Variabilität des Zellkern bei crepis - Arten in Bezug auf die Artbildung. Zeits. f. Zellforsch. u. Mikr Anat. 4: 171-215.
- Naithani, S.P. 1941. Cytological studies on Indian pulses, Part I. The somatic chromosomes and the Prochromosomes of Cajanus. Prog. Nat. Acad. Sci. India, 11.
- Natarajan, A.T. and M.D. Upadhyay. 1964. Localized chromosome breakage induced by EMS and hydroxyl amine in V. faba. Chromosoma, 15: 156-169.
- Natarajan, A.T. and G. Shivasankar. 1965. Studies on Modification of mutation response of barley seeds to ethyl methane sulphonate. Z. Vererbungsl., 96: 13-21.
- Nazeem, H.R., A.M. Hasan., H.S. Sherif., I.I. Elshawaf and M.M. El-Hady. 1933. Inheritance of some economic characters in lentil. In eight international Congress for statistics, computer science, social and demographic research held March 26-31, Egypt; Ahn. Shams Univ. Press 201-227.
- Newcomer, E.N. 1941. A colchicine induced tetraploid Cosmos J. Herd., 32: 161-181.
- Newman, L.J. 1966. Bridge and fragment aberrations in Podophyllum peltatum Genetics, 53: 55-63
- Nerkar, Y.S. 1977. Cytogenetical effects of gamma rays, Ethyl Methane Sulphonate and Nitrosomethyl urea in Lathyrus sativus. Ind. Jr. Genet. Pl. Breed., 37(1), 142-146.

- Nielson, E.L. and J. Nath. 1961. Somatic instability in derivatives of Agroelymum - turneri resembling Agropyron repens. Amer. J. Bot. 48: 345-349.
- Neguti, H. H. Oka and T. Otuka 1940. Studies in the polyploids of Nicotiana induced by the treatment with colchicine H. Growth and chemical analysis. Jap. Jk. Bot. 10: 343-364.
- Noggle, G.R. 1946. The physiology of polyploidy in plants. Lloydia, 9: 153-173.
- Ohno, R. and S. Tanikuzi, 1960. Cytological effects of extracts from noxious plants III. Meiotic abnormalities caused by water extracts from Arisaema japonicum. Jap. Jr. Genetics, 35: 167-176.
- Owen, F.W. 1928. Inheritance studies in soybean Genetics. 13: 50-79.
- Pathak G.N. 1948. Cytological studies of a spontaneously originated tetraploid Cajanus cajan Mill sp. Indian J. Genet., Pl. Breed., 9: 68-71.
- Panthau, J.V. 1967. Pachytene pairing and meiosis in the F₁ hybrid of Pennisetum typhoides and Pennisetum purpureum. Cytologia, 32: 532-541.
- Pal, M. and Khoshoo, T.N. 1977. Evolution and improvement of cultivated amaranthus VIII. Induced autotetraploidy in grain in types 2. Pflanzensucht. 78: 135-148.
- Patil, S. and J.K. Bhalla. 1985. Effects of Gamma irradiation on some malitative characters of soybeans. Ind. Jr. Bot., 8(2): 130-134.
- Pierce, W.P. 1937. The effect of phosphorus on chromosome and nuclear volume in a violet species. Bull. Torrey Bot. club., 64: 345-354.
- Powell, A.M. 1968. Chromosome number in perityle and related genera (Peritylanese - compositae) Amer. J. Bot., 55: 820-828.

Prasad, A.B. 1965. Studies in the development of irradiated seeds of Phaseolus canariensis and P. Minor. Ph. D. Thesis, Univt. of London.

Premasekar, S., Appadurai, R. 1981. Effect of doses of gamma rays and ethylmethane sulphonate on the germination and survival of induced mutations in Pigeon pea. Indian Jr. Agric. Sci., 51: (6): 381-386.

Pundir, R.P.S. 1981. Relationships among Cajanus, Atylosia and Rhynchosia species. Ph. D. thesis, Banarus Hindu University, Varanasi, India.

Pundir, R.P.S. and R.B. Singh 1985 a . Cytogenetics of P_1 hybrids between Cajanus and Atylosia species and its phylogenetic implications. Theor. Appl. Genet., 71: 216-220.

Pundir, R.P.S. and R.B. Singh 1985b. Biosystematic relationships among Cajanus, Atylosia and Rhynchosia species and evolution of Pigeon Pea (Cajanus cajan (L.) Mill. sp.). Theor. Appl. Genet., 69: 531-534.

Pundir, R.P.S. and R.B. Singh 1985 c. Crossability relationships among Cajanus, Atylosia and Rhynchosia species and detection of crossing barriers. Euphytica., 34: 303-308.

Randolph, L. P. 1935. Cytogenetics of tetraploid maize. J. Agric. Res., 50: 591-605.

Ramanujan, S. and Joshi, A.B. (1941). Colchicine induced polyploidy in crop plants I. Gram (Cicer arietinum L.) Ind. Jr. Agric. Sci. II. 835-849.

Rajhathy, T. and J.W. Morrison. 1960. Genetic homology in the genus Avena canad. Jr. Genet. cyt. 2: 278-285.

Raghuvanshi, S.S. and V.K. Chauhan. 1970. Quoted by A.K. Singh in cytogenetical studies in special reference to induction of polyploidy and Mutation. Ph. D. Thesis. Lucknow Univt.

Rao, D. Manohar and Turmala P. Reddy. 1985. Induction of Morphological Mutants in Cajanus cajan (L.) Mill sp. Ind. Jr. Bot., 8(2) : 198-202.

- Rees, H. and J.B. Thomson. 1955. Localization of chromosome breakage at Meiosis. Hereditas, 21: 399-407.
- Rees, H. 1961. Genotypic control of chromosome forms and behaviour Bot. Rev. 27: 288.
- Reddy, L.J. 1973. Inter-relationships of Cajanus and Atylosia species as revealed by hybridisation and pachytene analysis. Ph. D. Thesis, Indian Institute of Technology, Kharagpur, India.
- Reddy, R.P. and G.P. Rao, 1975. Somatic variation in Cajanus cajan. Curr. Sci. 44 (22): 816-817.
- Reddy, L.J. Green, J.M., Singh, U., Bisen, S.S., and Jambunathan, R. 1979. Seed protein studies on Cajanus cajan, Atylosia spp. and some hybrid derivatives. Page 105-117 in Proceedings, symposium on seed protein improvement in cereals and Grain Legumes. Vol. 2. International Atomic Energy Agency, Vienna, Austria.
- Reddy L.J. Green J.M. and D. Sharma 1980. Genetics of Cajanus cajan (L.) Mill sp. x Atylosia spp. in: ICRISAT 1981. Proc. Int. Workshop Pigeon pea, Patancheru, A.P., India.
- Remanandan, P. 1980. The wild gene pool of Cajanus at ICRISAT, present and future Vol. 2. Proceedings of the international workshop on Pigeon peas, ICRISAT, pp. 29-38.
- Reddy, L.J. 1981 a. Pachytene analysis in Cajanus cajan, Atylosia lineata and their hybrid Cytologia, 46: 397-412.
- Reddy, L.J. 1981b. Pachytene analysis in Atylosia sericea and Cajanus cajan x A. sericea. Cytologia, 46: 567-577.
- Reddy, L.J. 1981 c. Pachytene analysis in Atylosia scarabaeoides and Cajanus cajan x A. scarabaeoides hybrid. Cytologia, 46: 579-589.

- Reddy, L.J. and D.N. De. 1983. Cytomorphological studies in Cajanus cajan x Atylosia lineata. Ind. Jr. Genet., 43: 96-103.
- Rieger, R. and A. Michaelis. 1960. Chromatide seperrationen nach Einwirkungren Althyl - Methanu - ulphonate auf. Primarwurzelen Von vicia faba. L. Kulfurpflanze, 8 230-243.
- Riley, R. 1960. The diploidation of polyploid wheat, Heredity, 15: 407-429.
- Rieley, R. and C.N. Law. 1965. Genetic variation in chromosome pairing. Adv. Genet., 13: 57-144.
- Rieley, R., V. Chapman and A.M. Selfeld. 1966. Induced Mutation affecting the control of Meiotic chromosomal pairing in Triticum aestivum. Natur., 211: 368-369.
- Roy, Basudeo. 1933. Studies in the development of female gametophyte in some leguminous crop plants of India. Ind. Jour. Agric. Sci., 1098-1107.
- Roy Tapadar, N.N. 1963- Studies in induced tetraploids of family Apocynaceae. I. Rauvolfia serpentina Penth. Cytologia, 28 (3): 229-241.
- Roy, Ashok and D.N. De. 1965. Intergeneric hybridization of Cajanus cajan and Atylosia . Sci. Cult., 31: 93-95.
- Ruttle, M.L. and Nebel, B.R. 1939. Cytogenetic results with Colchicine biol. Zentralbl., 59: 79-87.
- Sax, K. 1940. An analysis of X-ray induced chromosomal aberrations in Tradescantia. Genetics, 25: 41-68.
- Sax, K. 1941. The behaviour of X-rays induced chromosomal aberrations in Allium root-tip cells. Genetics, 26: 418-425.
- Saunders, A.R. 1959. Inheritance in the cowpea (Vigna sinensis Endl.) I. colour of seed coat colour. Agg. Jr. Agr., 2: 285-307.

- santos, I.S. 1969. In proc. Symp. IAEA - F.A.O. (Wash): 169-179.
- sagar, P. and S. Chandra. 1980. Breeding behaviour and genetic variation for yield in crosses on lentil (Lens esculenta M.) Ind. Jr. Ag. Res., 14: 159-163.
- Schwanitz, P. 1948. Untersuchungen an polyploiden Pflanzen. I. Felclversuche Mit diploiden und autotetraploiden Pflanzen. Zuchter, 19: 70-88
- Schwanitz, P. 1950. VI. Pollen grosse und Zellengrosse bei diploiden und tetraploiden pflanzen. Zuchter, 20: 53-57.
- Schertz, K.F. 1962. Cytology, fertility and grass morphology of induced polyploids of Sorghum vulgare. Cand. J. Genet. Cytol., 4: 179-186.
- Sen, N.K. and H.R. Chheda 1958. Colchicine induced tetraploids of five varieties of black gram. Ind. Jr. Genet. Plant Breed., 19: 238-248.
- Sen, N.K. and A.K. Ghosh. 1960. Interspecific hybridization between Phaseolus aureus Roxb. (Green gram) and P. mungo L. (Black gram). Bull. Bot. Soc., Bengal, 14: 1-4.
- Sen, S.K. and C.B. Tiwari. 1966. A comparative Karyotype study of five varieties of pea (Pisum sativum L.) Nucleus, 11: 173-176.
- Selim, A., M.A. Hussein and E. Shawf I.I. S. 1974. EMS and X-ray induced mutations in Pisum sativum II. Effect of EMS and X-rays on M₁ generation seedling height and fertility. Egypt. J. Genet. Cyto., 3: 172-192.
- Sharma, A.K. 1956. Chromosomal studies in some Indian Barley. Proc. Nat. Inst. Sci. India, 22B: 246-254.
- Sharma, A.K. and D. Bhattacharjee. 1957. Chromosome studied in Sorghum. Cytologia, 22: 287-311.
- Sharma, A.K. and A. sharma. 1959. Recent Advances in the chromosome alterations with relation to speciation Bot. Rev., 25: 514-544.

- Sharma, B. 1966. A comparison of Mutagenic action of N-nitroso methyl urea with various Physical and chemical mutagens in garden peas in supermutagens, Nauka, Moscow, 143-159.
- Shinde, V.K., R. D'cruz, and A.B. Decker, 1971. Genetic studies in pigeon peas. XI. Creeping 3-28 x red grained. Poona Agric. Coll. Mag. 61: 53-55.
- Shaikh, M.A. Q and M.B.E. Godward, 1972. The mitotic consequences of radiation induced chromosome breaks in Lathyrus sativus and vicia ervilia. Cytologia, 37:(3), 489-495.
- Shaikh, M.A.Q. and M.B.E. Godward, 1972. The meiotic consequence of radiation induced chromosomes break in lathyrus sativus and vicia faba. Cytologia, 37(3): 497-505.
- Shrivastava, M.P., D. Sharma, and Laxman singh. 1973. Karyotype analysis in 15 varieties of Cajanus cajan (L.) Mill sp. and Atylosia lineata (W and A). Cytologia, 38 (2): 210-227.
- Sharma, A.K. 1977. Evidence of dynamisms as a basis of chromosomal control of genetic reactions. Nucleus, 20: 4-10.
- Sharma, S.K. and B. Sharma 1978a. Induction of tendril Mutations in lentil (Lens culinaris Medik.) Curr. Sci., 47, 22: 864-866.
- Sharma, S.K. 1978 b. Induced variability for pod and seed size in lentil (Lens culinaris Medik). Curr. Sci., 47 (21): 806-807.
- Sharma, S.K. 1979b. Leaf mutations induced with NMU and gamma rays in lentil (Lens culinaris Medik.) Curr. Sci., 48 (20): 916-917.
- Sharma, A. (1985). Chromosomes IInd ed; Oxford and IBH Publishing company.
- Sikdar, A.K. and De, D.N. 1967. Cytological studies of two species of Atylosia and Cajanus cajan. Bulletin of the Botanical Society of Bengal, 21(1): 25-28.

- Sikka, S.M., Mehta R.K and Swaminathan, M.S. 1959. studies on induced polyploids in forage crops. II. Colchicine treatment methods for berseem and senjii. Indian Jr. of Genet., 19: 90-97.
- Singh, R.P. and J.E. Gunkel. 1965. Rad. Bot., 5: 525-542.
- Sikdar, A.K and D.N. De. 1967. Cytological studies of two species of Atylosia. Bull. Bot. Soc. Bengal, 21(1):
- Siddiq, E.A. 1967. Studies on the induction of polyploids in Maize and Sorghum and on the elimination of diploid cells in colchicine treated Maize. M. Sc. Thesis I.A.R.I. New Delhi.
- Sikdar, A.K., and D.N. De. 1967. Cytological studies of two species of Atylosia and Cajanus cajan. Bull. Bot. Soc. Beng., 21 (1) : 25-28.
- Sigurbjornsson, B. and A. Mücke. 1969. Progress in Mutation breeding. Proc. Symp. Induced mutations in plants, Pullman, 673-698. IAEA/PAO, Rome.
- Sinha, S.S.N. and M.B.E. Godward. 1969. Radiation studies in Lens culinaris. Distribution of chiasmata between nuclei and with nuclei in irradiated and non-irradiated populations. Cytologia, 34: 45-51.
- Sinha, S.S.N. and M.B.E. Godward, 1972. Radiation studies in Lens culinaris. Meiosis: abnormalities induced due to gamma radiation and its consequences. Cytologia 37: (4) 685-695.
- Sinha, S.S.N. and M.B.E. Godward. 1972. Radiation studies in Lens culinaris. Ind. Jr. Genet. Pl. Breed., 32 (3) 331-339.
- Sinha, S.S.N. and S.S. Accharia. 1974. Cytological studies in Lens nigricans. A case of translocation heterozygote. Cytologia, 39: 57-62.
- Sinha, S.S.N., 1979. Mitotic analysis in thirteen varieties of Cajanus cajan (L.) Mill sp. Cytologia, 44: 571-580.

- sinha, S.S.N. and P. Kumar. 1979. Mitotic analysis in thirteen varieties of Cajanus cajan (L.) Mill sp. Cytologia. 571-580.
- Singh, V.P., R.D. Yadav, I.P. Singh and R.M. Singh. 1984. Induction of Morphological variation and some mutations in green gram by gamma rays. National Acad. Sci. Ind. IV. Sec. (B), Part 2: 81-87.
- Sparrow, A.H., W.L. Ruttle and B.R. Nebel. 1942. Comparative cytology of sterile intra and fertile inter-varietal tetraploids of Antirrhinum majus L. Amer Jr. Bot. 29 : 711-715.
- Stadler, L.J. 1931. The experimental modification of heredity in crop plants I. Induced chromosomal irregularities. Sci. Agr. 11: 557-572.
- Stebbins, G.L. 1938. Genetics, 23: 83.
- Strand, A.B. 1943. species crosses in the genus phaseolus. Proc. Amer. Soc. Hort. Soc., 42: 469-573.
- Stebbins, G.L. 1945 a. The cytological analysis of species hybrids II. Bot. Rev., 11: 463-486.
- Stebbins, G.L. 1950. Variation and evolution in plants. Edward Arnold Ltd. 41 Maddoxstreet. London.
- Stebbins, G.L. 1971. Chromosomal evolution in higher plants. Edward Arnold Ltd. 41. Maddoxstreet, London.
- Swaminathan, M.S. and B.R. Murthy. 1957. One way incompatibility in some species crosses in genus. Nicotiana. Indian J. Genet. 17: 23-26.
- Swanson, C.P. and R. Nelson. 1942. Spindle abnormalities in Mentha. Bot. Gaz., 104: 273-280.
- Swaminathan M.S., V.L. Chopra and S. Bhaskaran. 1962. Chromosome aberrations and the frequency and spectra of mutation induced by EMS in barley and wheat. Ind. Jr. Genet. 22: 192-207.

APPENDIX

A list of the Scientific Research papers accepted for publication is as follows:

1. Kalpana Srivastava and S.N. Tripathi, 1985.
Cytomorphological studies in Atylosia lineata,
Atylosia caianifolia and their F_1 hybrid.
Jr. Ind. Bot. Soc. (In press).
2. Kalpana Srivastava and S.N. Tripathi, 1986.
Interspecific cross between Atylosia platycarpa
(Benth) and Atylosia mollis (Benth). Ag. Sci.
Dig. (In press).
3. Kalpana Srivastava and S.N. Tripathi, 1986.
Cytomorphological studies on induced tetraploids
of A. scarabaeoides and Atylosia platycarpa.
Forage Research (In press).
4. Kalpana Srivastava and S.N. Tripathi, 1986.
Cytomorphological observations in Atylosia
lineata x Atylosia albicans. Legume Research
(In press).

The experimental part of the research work accepted for publication in joint authorship, has been done entirely by the senior author.